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## Evaluation of dicamba volatility when applied under field and controlled environmental conditions

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Evaluation of dicamba volatility when applied under field and controlled environmental  
conditions

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A Thesis  
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Mississippi State University  
in Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
in Agronomy  
in the Department of Plant and Soil Sciences

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Dicamba resistant (DR) cropping technology has increased dicamba use, resulting in observation of dicamba off-target-movement (OTM). Volatility is one form of this movement. Tank mixtures and environmental conditions impact the volatile behavior of dicamba following application. Research was conducted in 2018, 2019, and 2020 to further assess and understand volatility mitigation by understanding tank-mix effects and utility of irrigation on volatility mitigation. Low tunnel and humidome methodology were used to analyze impact of tank mixtures and irrigation on dicamba volatility. Data suggest tank mixing encapsulated chloroacetamide formulations can mitigate volatility when comparing identical active ingredients formulated as emulsifiable concentrates. Tank-mixed glyphosate increases dicamba volatility regardless of salt form, with dimethylamine salt of glyphosate having the most volatile effect. Manipulation of environmental conditions can also assist in mitigation efforts when applicable through use of irrigation. Increasing amount of irrigation applied following dicamba application has a positive effect on mitigation.

## DEDICATION

I would like first to acknowledge my parents for their impact on my completion of this graduate degree. Without the push from both my parents – Marje and Rob Taylor – there is no chance I would be where I am today. Their support and guidance through this experience has been invaluable. I would also like to thank Gabrielle Fuller for keeping me sane through the process when deadlines seemed daunting.

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## CHAPTER I

### INTRODUCTION

Weeds have remained a top pest in agricultural production for much of the modern agricultural era (Burnside 1993). With current commodity prices, many of today's farming operations consist of large acreages with slim profit margins and few employees. To remain profitable and maintain high production levels, methods of weed management primarily consist of chemical methods (Derpsh and Friedrich 2009, Givens et al. 2009). Chemical weed control methods provide economical and efficient control, attainable with reduced labor (Gianessi and Reigner 2007). Although efficient, chemical control increases selection pressure upon weed species and promotes herbicide resistance worldwide (Vencill et al. 2012). Herbicide resistance among numerous weed species threatens agricultural productivity and producer profit. Herbicide tolerant cropping technology developments have been released to assist in the management of herbicide resistant weed species. Advancement in cropping technology has promoted the use of fewer modes of action and changed weed management tactics, thereby increasing herbicide selection pressure and instances of herbicide resistant weeds (Vencill et al. 2012, Givens et al. 2009).

Glyphosate was formerly the most important herbicide for agricultural weed control (Powles 2008). In 1996, glyphosate resistant (GR) cropping systems became available, enabling POST application of glyphosate in several major row crops (Gianessi 2005). These applications controlled the gambit of weed problems a producer could face in their field at the time the

technology was released. Economical application costs paired with efficacy over many species promoted immediate widespread adoption (Green and Owen 2011, Young 2006). With GR varieties available in corn, cotton, and soybean; selection pressure increased as glyphosate application frequency increased (Powles 2008, Johnson et al. 2010).

Today, producers are suffering the effects of the exclusive glyphosate weed management systems of the past, with a growing list of 17 glyphosate resistant (GR) weed species in the United States (Heap and Duke 2018). Accompanied by little herbicide development since the early 2000's, the list of herbicides used to control GR weed species POST is short and easily overused (Ruegg et al. 2007). Transgenic crop technology, allowing the use of older existing herbicides, became one solution for weed management issues related to GR weeds. The development of crops allowing postemergence use of the auxin dicamba was one such solution. This technology is known as the dicamba tolerant (DT) cropping system.

The DR cropping technology provides producers with the ability to apply dicamba POST in conjunction with additional broad spectrum active ingredients, such as glyphosate and glufosinate (Bayer Crop Science 2021). With GR weeds becoming more widespread and glufosinate applications more costly (Culpepper et al. 2000), dicamba was thought to be the “silver bullet” that producers had been waiting on (Behrens et al. 2007). Dicamba provides effective control of many GR *Amaranthus spp.* and many other GR broadleaf weeds (Johnson et al. 2010). Following the path of previously released crop technology, adoption occurred quickly (Weschler et al. 2019). With increased adoption came increased application frequency (Weschler et al. 2019).

Dicamba, a Weed Science Society of America (WSSA) group 4 herbicide available in various salt formulations, belongs to the synthetic auxin herbicide family and is classified as a

benzoic acid (Bunch et al. 2012, Hartzler 2017). Mimicking hormonal properties of natural auxins present in plants (Grossman 2010), uptake occurs naturally. Once uptake occurs, unnaturally high levels of auxins present in plant tissue causes abnormal growth patterns (Grossmann 2010, Bunch et al. 2012). Phytotoxicity in susceptible plants following exposure is observed as epinastic growth, leaf curling, node stacking, and other forms of unnatural growth (Behrens and Leuschen 1979). Although off target movement (OTM) is not new with dicamba (Auch and Arnold 1978, Behrens and Leuschen 1979, Boerboom 2004), increased usage has raised concern from producers, registration agencies, and environmentalist about dicamba fate after application.

Herbicide OTM can occur as physical drift, tank contamination, and vapor movement (Soltani et al. 2020, Behrens and Leuschen 1979, Boerboom 2004). Physical drift is the movement of herbicide spray solution at the time of application by wind (Soltani et al. 2020), referring to individual spray droplets as driftable fines (Creech et al. 2015). Driftable fines are defined as spray droplets measuring less than 200 microns and are more likely to drift when compared to larger droplets (Womac et al. 1997). Tank contamination results from residual herbicide molecules present in the spray tank, hoses, and pumps of spray equipment following proper cleanout procedure (Boerboom 2004). Residual herbicide contained in the sprayer is then potentially applied during the next spray event on non-target species. Vapor movement, or volatility, is the movement of pesticide vapors as a gas or fumes by wind (Maybank 1978, Grover 1975). Vapor movement occurs after the initial application resulting from the transition of liquid herbicide solution or solids into a gaseous state (Behrens and Leuschen 1979). Gaseous vapors are then moved to neighboring crops via wind, potentially causing undesirable response from non-tolerant plants.

The acute effects of dicamba OTM requires attention and mitigation efforts from all applicators. Physical dicamba drift can be mitigated at the time of application through use of drift reduction agents (DRA), drift mitigating nozzles, reduced ground speeds, and application during optimal winds (Egan and Mortenson 2012, Dexter 1993). Crop injury due to tank contamination may be mitigated through segregation of spray equipment by crop technology or use of hose material with less dicamba sequestration potential coupled with a triple rinse cleanout system (Cundiff et al. 2017). Volatility is a complex phenomenon with the potential to occur long after applications, making mitigation difficult (Mueller et al. 2013, Behrens and Leuschen 1979). Frequently changing environmental conditions, such as air temperature, relative humidity, wind, and rainfall, have impacts on the severity and likelihood of OTM as volatility (Behrens and Leuschen 1979, Hartzler 2017). With a lack of environmental control by applicators and potential for long-range vapor travel, volatility can potentially become a risk to susceptible crops long after the application and over long distances with no current mitigation efforts possible following application. Efforts to mitigate dicamba volatility include the use of new formulations and the use of volatility reduction agents (VRA).

Non-tolerant plant sensitivity makes understanding dicamba's OTM imperative to environmental and neighboring crop stewardship. The observations of dicamba OTM stems from the sensitivity of non-tolerant crops to minute concentrations (Hartzler 2017). For comparison, glyphosate use rates of 1% the labeled rates cause visible injury to *Zea mays* L. while 0.005% of labeled use rates of dicamba are required to observe soybean injury (Hartzler 2017). Soybean is among the most sensitive non-target crop potentially affected by dicamba OTM (Sciumbato et al. 2004, Jones et al. 2019). Soybean has shown visible injury at dicamba application rates of 0.028 g ae ha<sup>-1</sup> (Solomon and Bradley 2014). Research has shown yield reductions with rates as low as

0.04 g ae ha<sup>-1</sup> in susceptible soybean plants (Weidenheimer et al. 1989). However, identifiable dicamba injury can occur at rates lower than required for yield loss (Auch and Arnold 1978, Kelley et al. 2005, Wax et al. 1969). Timing of dicamba exposure also plays a role in the extent of phytotoxic response observed in non-tolerant crops. Injury ratings of 8% in soybean at the V2 stage resulted in a 10% yield reduction, while 2% injury ratings resulted in a 10% yield loss at the V5 and R2 soybean stage (Robinson et al. 2013). Susceptible soybean sensitivity to dicamba OTM is most sensitive between the V4 to R2 growth stage (Scholtes et al. 2019).

Understanding of the impacts of OTM has led to numerous regulations on dicamba application. Calendar date cutoffs regarding dicamba application have been implemented in certain states to lessen the percentage of soybean and other crops at more-sensitive growth stages in these states when dicamba is being applied (Redbond 2017). In Mississippi, only certified pesticide applicators can legally apply dicamba products labeled for POST crop applications in accordance with dicamba product label (Mississippi State University Extension 2018). All applicators and purchasers are required to complete a dicamba applicator course and purchaser training annually to increase stewardship awareness (EPA 2020, Mississippi State University Extension 2018). Applicator training reinforces and makes applicators aware of the ever-changing regulations, including buffer requirement, appropriate wind speed range, legal tank-mixed products, and ground speed limits that exists for legal dicamba application (Anonymous 2020a, Anonymous 2020b, Anonymous 2018). Record keeping requirements were also implemented to attempt to track and pinpoint sources of dicamba OTM (EPA 2020).

Environmental Protection Agency records require documentation of formulation, awareness of sensitive crop direction, nozzle, tank mixture, time of application, wind speed, and a variety of other OTM factors (EPA 2020). Despite regulatory methods to mitigate dicamba OTM,



increased dicamba use has resulted in widespread soybean hectares exhibiting dicamba injury from OTM (Bradley 2017, Hager 2017, Steckel et al. 2017).

Although efforts by applicators can help mitigate dicamba's vapor movement through actions and efforts taken, the chemical's characteristics have strong determination in its volatile behavior regardless of external forces (Hartzler 2017). Dicamba's volatility is related to its high vapor pressure (Grover 1975, Hartzler 2017). Volatility concern in herbicides begins in chemistries exhibiting vapor pressures above  $1.3 \times 10^{-5}$  Pa (Ross and Lembi 1999). Little to no volatility is observed in herbicides expressing vapor pressures below  $1.0 \times 10^{-4}$  or  $1.0 \times 10^{-3}$  Pa (Guth et al. 2004). Dicamba acid, the herbicidally active form of dicamba, has a vapor pressure of  $4.5 \times 10^{-3}$  Pa, well above the volatility threshold and showing potential concern for likelihood of volatility (Bunch et al. 2012). However, when formulated as a salt, vapor pressure of the chemical is far below the acid form (Behrens and Leuschen 1979).

Modern dicamba herbicides labeled for use in DT crops are formulated as salts (Anonymous 2020a, Anonymous 2020b, Anonymous 2018). Salt formulations aid in solubility, translocation, and adsorption of herbicides in water carriers (Travlos et al. 2017). Although also formulated as esters, salt formulations of dicamba are less volatile than other formulations (Egan and Mortensen 2012). A study in 1979 resulted in decreased dicamba vapor injury upon increasing NaCl concentration in spray solutions (Behrens and Leuschen 1979). Diglycolamine (DGA) with VaporGrip® and N,N-Bis-(3-aminopropyl) methylamine salt (BAPMA) salt formulations are less volatile when compared to other salt formulations of the herbicide (Jones et al. 2019, Egan and Mortensen 2012). Resulting from decreased quantified vapor and less volatility injury found in previous research, DGA and BAPMA salt formulations of dicamba are the only available formulations available for applications in DT cropping systems.

Highly volatile dicamba applications result from dissociation of dicamba from salt bonds (Behrens and Leuschen 1979). Dissociation of dicamba from salt formulation occurs in the presence of water in spray tanks or the environment (Abraham 2018, Zollinger 2018). After dissociation of the dicamba salt molecule, dicamba is free in solution and bonds with free  $H^+$  ions to create dicamba acid (Abraham 2018). Dicamba acid is more volatile than dicamba formulated as a salt (Behrens and Leuschen 1979). Formation of highly volatile dicamba acid with  $H^+$  ion bonding explains the direct relationship found between lower solution pH and increased volatility (Behrens and Leuschen 1979, Mueller and Steckel 2019a).

Certain tank additions have shown to affect the pH and vapor movement severity of dicamba herbicides (Mueller and Steckel 2019a, Mueller and Steckel 2019b). Numerous restrictions exist among tank-mix partners for dicamba applications to avoid antagonism and mitigate OTM (Anonymous 2020a, Anonymous 2020b, Anonymous 2018). Additionally, VRA use is required for dicamba applications in DT crops, according to 2020 label revisions (Anonymous 2020a, Anonymous 2020b). VaporGrip® technology was introduced as the “in-the-jug” volatility mitigation adjuvant and is present in DGA formulations applied in DT crops (Anonymous 2020a, Anonymous 2018). VaporGrip® is designed to reduce the formation of free dicamba acid in spray solutions by acting as a buffering agent using acetic acid (Abraham 2018, MacInnes 2017). Acetic acid present in the technology binds up free dicamba ions dissociating in solution before bonding with  $H^+$  ion and dicamba acid formation can occur (Abraham 2018). A 2020 study found that VaporGrip® technology in tank mix with DGA salt of dicamba reduced volatility injury observed from DGA salt of dicamba alone and BAPMA salt of dicamba (Oseland et al. 2020).

Despite mitigation efforts and regulatory action, dicamba OTM has become a source of many lawsuits against the agrochemical company, Bayer. Since release, registration updates have occurred in 2018, 2019, and 2020 to input additional OTM mitigating regulation and provide additional OTM mitigating products for use with the technology (EPA 2020). In June of 2020, the Ninth Circuit Court of California ruled to vacate labels of the three dicamba products able for POST application in DT crops (EPA 2020). These products were Bayer's XtendiMax®, BASF's Engenia®, and Corteva's FeXapan® (EPA 2020). Loss of registration of these products stemmed from recurring injury being observed on non-target crops and trees. In October of 2020, registration of these products was extended by the EPA until 2025, barring further EPA action to amend registration (EPA 2020). The 2020 registration amendments further restrict use of the products in POST application and require use of additional OTM mitigating agents (EPA 2020).

With several factors affecting dicamba OTM, difficulty arises in narrowing down a singular method of dicamba OTM remediation. Volatility research is necessary to increase understanding of dicamba vapor behavior and mitigate occurrence. Further knowledge of factors and influencers of dicamba volatility, whether tank mixes, environmental conditions, or other application characteristic, can assist in the mitigation of volatility by direct influence of applicators and allow for continued registration and use of this effective weed management tool.

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## CHAPTER II

### DETERMINING EFFECT OF TANK MIXTURES OF CHLOROACETAMIDE HERBICIDES AND GLYPHOSATE ON DICAMBA VOLATILITY

#### **Abstract**

Soybean injury by off-target-movement (OTM) of dicamba has become more widespread since introduction of the dicamba-resistant cropping system. Volatility is one form of dicamba OTM. Volatility is affected by an array of diverse factors ranging from environmental conditions, tank-mix partners, and application timing. With increasing use of tank-mix partners with dicamba and use in pre-mixed products, further research of tank-mix effects on dicamba volatility is needed. Field and greenhouse experiments were conducted in 2019 and 2020 to evaluate the effects of common chloroacetamide tank-mix partners and glyphosate on diglycolamine (DGA) dicamba with VaporGrip® volatility. Experiments were conducted as a two-level factorial with Factor A levels consisting of dicamba alone, dicamba plus emulsifiable concentrate (EC) *S*-metolachlor, dicamba plus EC acetochlor, a premixed product containing dicamba plus capsule suspension (CS) *S*-metolachlor, and dicamba plus microencapsulated (ME) acetochlor. Factor B levels consisted of the presence or absence of K salt of glyphosate. Field treatments were applied at 4X labeled rates to greenhouse flats filled with soil wetted prior to application. Treated flats were placed between two rows of non-dicamba-resistant soybean at the center of each 15.3 m plot containing a 6.2 x 1.5 m low tunnel dome covered with plastic.

Visible injury (%) and plant heights (cm) were recorded in the most visibly injured quadrant every 30 cm 14 and 28 days after conclusion of the 48-hour exposure period. Each low tunnel contained an air pump sampling air through a polyurethane foam tube (PUF) to catch dicamba molecules that vaporize from the treated soil surfaces. Humidome treatments contained identical factors applied at labeled rates to greenhouse flats contained in humidome systems. Air samplers pulled air through the sealed system and PUF to capture and quantify dicamba volatility from treated soil. Field PUF data suggest separation in dicamba volatility is dependent upon chloroacetamide formulation in field settings, but no differences in chloroacetamide effects were found in humidome experiments. Tank-mixed glyphosate increased quantifiable dicamba volatility in both field and humidome PUF samples. The EC chloroacetamide formulations were found to increase extent and distance of volatility injury when compared to non-EC formulations of the same active ingredient 14 days after treatment (DAT). Glyphosate increased vapor injury severity and distance when tank mixed at both rating timings. No effect on plant height was observed between factors or as main effects.

**Nomenclature:**

Acetochlor; diglycolamine salt of dicamba; potassium salt of glyphosate; *S*-metolachlor; soybean, *Glycine max* (L.) Merr.

**Key Words:**

Emulsifiable concentrate, microencapsulated; capsule suspension; volatility.

## Introduction

Since release of the dicamba-resistant (DR) cropping system, POST use of dicamba has increased (Wechsler et al. 2019, Werle et al. 2018). The ability to manage problematic glyphosate-resistant (GR) weed species with dicamba has resulted in rapid adoption of the technology since its 2016 release (Shergill et al. 2018, Wechsler et al. 2019). Between 2016 and 2018, DR soybean hectares increased 43% nationally (Wechsler et al. 2019). Areas with increased observance of GR weed species have seen widespread adoption of DR soybean (Wechsler et al. 2019). In the 2018 Mississippi cropping season, 79% of the soybean hectareage was planted with DR soybean (Wechsler et. al 2019). Alongside increased adoption came an increase in dicamba application frequency during the cropping season (Wechsler et al. 2019). Although not every hectare of DR soybean receives a POST application of dicamba, the option is available and utilized by many growers (Wechsler et al. 2019). In the 2018 Mississippi soybean crop, approximately 54% of the DR soybean hectares received a dicamba application (Wechsler et al. 2019). Increased dicamba application frequency has resulted in increased dicamba injury from off-target-movement (OTM) (Bish and Bradley 2017, Mueller and Steckel 2019).

Dicamba OTM occurs as physical drift, tank contamination, and vapor movement (Soltani et al. 2020, Behrens and Leuschen 1979, Boerboom 2004). Mitigation efforts of physical drift include use of larger droplet size, use of hooded-sprayer design, use of drift reduction agents, reduction of ground speeds, and making applications under favorable weather conditions (Foster et al. 2018, Creech et al. 2015, Womac et al. 1997). Dicamba contamination of spray equipment can be mitigated through segregation of spray equipment by herbicide technology, or selection of sprayer components less likely to sequester the herbicide (Cundiff et al. 2017). Volatility mitigation is attempted through understanding impacts of application timing,

understanding of ever-changing environmental conditions at and following application, and understanding of tank mixture effects (Behrens and Leuschen 1979, Mueller and Steckel 2019). The inability to control environmental conditions after application (Mueller et al. 2013), makes tilting the pendulum of dicamba volatility mitigation through tank mix crucial for successful efforts.

Understanding tank-mixing effects allows for applicator-controlled mitigation efforts of dicamba volatility before the sprayer enters the field. Tank-mixing has become a popular method to mitigate resistance development, broaden spectrum of control, and reduce the number of applications (Beckie and Reboud 2009, Norsworthy et al. 2012). With dicamba's lack of activity on grass species and limited residual activity, POST dicamba applications routinely include a tank-mix partner with grass and residual activity (Werle et al. 2018, Spaunhorst and Bradley 2013). With DT soybean systems also exhibiting glyphosate tolerance, glyphosate is frequently included for additional control of susceptible broadleaf species and grasses (Werle et al. 2018). In a 2018 survey in Nebraska, 60% of producers applied dicamba alone or with glyphosate POST, while 40% applied dicamba with herbicides of different modes of action (Werle et al. 2018). Alongside tank-mixed herbicides having foliar activity, soil applied residual herbicides are recommended in POST applications to increase length of control or provide additional control as initial residual control lessens (Norsworthy et al. 2012). Impacts of these residuals on dicamba volatility must be understood for effective stewardship of the environment and neighboring crops.

Diverging from trends beginning in 1996 of decreasing MOA diversification and little residual herbicide use, today's producers are more aware of utilizing additional chemistries to mitigate POST herbicide selection pressure (Beckie et al. 2019, Bonny 2016). Group 15

chloroacetamide herbicides remain popular for residual control in soybean and *Gossypium hirsutum* L. (Butts et al. 2019). Chloroacetamide herbicides are used POST to provide residual soil activity and increase control of GR weeds along with foliar POST herbicides (Clewis et al. 2006, Cahoon et al. 2015, Jhala et al. 2015). Group 15 herbicides work by inhibiting biosynthesis of very long chain fatty acids and other enzymatic reactions within the plant (Fuerst 1987, Matthes et al. 1998). Without successful biosynthesis of fatty acids, cell membrane structure and permeability are lost, and plant death occurs (Matthes and Boger 2002).

Common examples of herbicides in the chloroacetamide family used in soybean and other crops are *S*-metolachlor and acetochlor. *S*-metolachlor and acetochlor were the third and fourth most applied herbicides behind glyphosate and atrazine in 2008 (Fernandez-Cornejo et al. 2014). These molecules control a variety of grasses, broadleaf, and sedge weed species and are available in a variety of formulations and pre-mixes (Anonymous 2012, Anonymous 2015, Anonymous 2019). Many problematic weed species of Mississippi and the surrounding regions, such as Palmer amaranth (*Amaranthus palmeri* S. Watson), waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), and Italian ryegrass (*Lolium multiflorum* Lam.), are controlled by these molecules (Whitaker et al 2010, Steckel et al. 2002, Bond et al. 2014).

Selection pressure due to increased usage of auxin herbicides is resulting in increased concerns of auxin resistance development (Busi et al. 2018). DR populations of wild mustard (*Brassica kaber* (D.C.) L.C. Wheeler) and kochia (*Kochia scoparia* (L.) Schrad.), have been found in regions of the United States with frequent dicamba use (Cranston et al. 2001, Jasieniuk et al. 1995). Loss of dicamba efficacy to additional weed resistance developments could make targeting problematic broadleaf weeds in DR cropping systems more difficult. To mitigate the development of resistant populations, weed scientists recommend the use of multiple

modes of action within single applications (Norsworthy et al. 2012). In tank mixtures with glyphosate, *S*-metolachlor was found to decrease risk of glyphosate resistance in Palmer amaranth to 12%, compared to 74% with glyphosate alone (Neve et al. 2011). Tank mixes containing chloroacetamide herbicides can increase length of control from dicamba applications while mitigating long-term development of additional DR weed populations. The frequency of tank mixing chloroacetamide and glyphosate herbicides in POST applications commonly containing dicamba creates the need to understand impacts of these chemistries on dicamba volatility.

## **Materials and Methods**

In 2019 and 2020, experiments were conducted in Starkville, MS, at the R.R. Foil Experiment Station and Brooksville, MS, at the Black Belt Research Station to evaluate the effects of various chloroacetamide herbicides and glyphosate on dicamba volatility. Experiments were conducted using a randomized complete block design with a factorial arrangement of treatments. Factor A consisted of a 4X rate of various chloroacetamide herbicides, while Factor B was the presence or lack of glyphosate within the spray mixture. Plots measured 15.3 m x 0.8 m, with 3 replications separated by alleys measuring 6.1 m. Two unplanted rows of soybean measuring 2.3 m in width separated plots in the same replication to mitigate potential vapor movement between treatments. Two rows of 'CZ 4539 GTLL' soybean were planted with a seeding rate of 345,940 seeds ha<sup>-1</sup> in each plot as indicator plants. Treatments occurred between the V4 and V5 vegetative growth stages to ensure dicamba exposure prior to the initiation of reproductive structures. Treatments were applied to greenhouse flats (Heavy Duty 1020 Tray, Greenhouse Megastore) containing field soil saturated the night before application. Soil-filled greenhouse flats were weed free and uniform in soil surface texture to create a uniform

application surface. Applications in the Brooksville location were conducted using a CO<sup>2</sup> propelled backpack sprayer with a carrier volume of 140 L ha<sup>-1</sup>. Treatments were made with a single application at 4X labeled rates to ensure phytotoxic response of indicator soybean. Applications in Starkville were conducted using an enclosed track sprayer with an identical carrier volume and application rate as the Brooksville location. Soil flat application occurred in a separate location from field experiments in both locations and were transported to the experiment site via truck bed.

Factor A levels contained emulsifiable concentrate (EC) *S*-metolachlor applied as Dual Magnum® (Syngenta, Greensboro, NC) at 4.4 kg ai ha<sup>-1</sup>, microencapsulated (ME) acetochlor applied as Warrant® (Bayer Crop Science, St. Louis, MO) at 5.06 kg ai ha<sup>-1</sup>, a capsule suspension (CS) pre-mix of diglycolamine (DGA) salt of dicamba plus *S*-metolachlor applied as Tavium plus VaporGrip® (Syngenta, Greensboro, NC) at 6.69 kg ai ha<sup>-1</sup>, EC acetochlor applied as Harness® (Bayer Crop Science, St. Louis, MO) at 5.04 kg ai ha<sup>-1</sup>, and no chloroacetamide addition. Factor B levels consisted of the addition of potassium salt of glyphosate (RoundUp PowerMax®, Bayer Crop Science, St. Louis, MO) applied at 3.47 kg ae ha<sup>-1</sup> and no glyphosate addition. Each treatment contained DGA salt of dicamba with VaporGrip® (Bayer Crop Science, St. Louis, MO) applied at a rate of 2.24 kg ae ha<sup>-1</sup> except for treatments containing the pre-mix formulation in Tavium plus VaporGrip®. Solution pH of each treatment was taken after application.

Following herbicide application, greenhouse flats containing treated soil were placed in the center of each experimental unit. A 1.5 m x 4.6 m PVC frame was placed in the center of each experimental unit (Figure 2.1). Contractor's plastic was draped over the PVC structure and clamped on both ends (Figure 2.2). The ends of the low tunnel remain open to allow for air

movement through the tunnel. Treated soil and low tunnels remained in each plot for a 48-hour treatment period. Following that period, all low tunnels, contractor's plastic, and soil flats were removed from the field.

Collection of visible injury and plant heights occurred 14 and 28 days after treatment (DAT). Evaluations were based on the conclusion of the 48-hour exposure period. One to two days prior to the first evaluation, the most injured quadrant from each plot was identified. Ratings occurred within this quadrant at both evaluation intervals. Ratings in the selected quadrant occurred outward from the center of the plot in 30 cm intervals. Plant injury ratings used a percentage scale from 0 to 100% as a percentage of injury compared to the untreated check (Behrens and Leuschen 1979). Plant heights were collected in centimeters at each evaluation.

Air sampling occurred at the center of each plot. A low-volume SKC polyurethane foam tube (PUF) (SKC, Eighty-Four, PA) was positioned 30.5 cm above treated soil flats to quantify dicamba molecules volatilizing. PUFs were connected to an SKC AirChek 52® (SKC, Eighty-Four, PA) air sampler calibrated to pull air through the PUF at a rate of three Liters per minute. Air sampling occurred for the entirety of the 48-hour incubation period with PUFs collected at its conclusion. Analysis of PUF concentration was conducted by the Mississippi State Chemical Lab using liquid chromatography/mass spectrometry to analyze concentrations of dicamba molecules of each PUF in nanograms.

Dicamba concentration was quantified using an Agilent 1290 liquid chromatograph coupled with an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed using an Agilent Zorbax Eclipse Plus 100 mm column. The mobile phases consisted of 0.1% formic acid in water for the aqueous



phase (A) and 0.1% formic acid in acetonitrile as the organic phase. The flow rate 0.3 mL/min with the following gradient program: 0 to 0.5 min of 25% B, 0.5 to 1 min of 50% B, and 1 to 4 min of 60% B. The ionization of dicamba was preformed using electrospray ionization (ESI) in negative mode with an auxiliary gas ( $N^2$ ), source temperature of 200°C, and a gas flow rate of 10 L/min.

Data were subjected to ANOVA to evaluate significance of main effects and interactions of factors. Injury and plant height evaluation at each collection distance used PROC GLIMMIX with means separated by LSMEANS using an alpha level of 0.05 in SAS 9.4® (SAS Institute Inc, Cary, NC). Percent injury, pH measurements, and PUF concentrations were analyzed over site year using PROC GLIMMIX and means separated by LSMEANS using an alpha level of 0.05 in SAS 9.4. Plant injury and plant height data were also nonlinearly regressed over site year with a 95% confidence band using the loess package in RStudio® (RStudio Inc, Boston, MA) due to non-parametric behavior of the data (Scholtes et al. 2019).

Complimentary humidome experiments were conducted in 2019 in a greenhouse located in Starkville at the R.R. Foil Experiment Station to evaluate the effects of various chloroacetamide herbicides and glyphosate on dicamba volatility under controlled environmental conditions. Experiments were conducted in a randomized complete block design with a factorial arrangement of treatments with three replications. Experimental units consisted of a greenhouse flat (Heavy Duty 1020 Tray®, Greenhouse Megastore) topped with an unvented humidity dome (7” Mini Greenhouse®, Mondri). Within each greenhouse flat, a smaller greenhouse flat (1010 Tray Insert, Greenhouse Megastore) filled with field soil was contained. Soil within the smaller greenhouse flat received the herbicide application.

On each end of the humidity dome, a hole was made through the plastic dome top. A 0.95 cm hole was made on ends closest to treated soil flats. On the opposing end of the dome, a 1.43 cm hole was made to allow for a threaded male fitting through the hole. On the male end of the fitting, located outside of the humidity dome, a 12.7 cm section of neoprene hose was attached to the fitting to allow PUF and air sampler attachment. An SKC AirChek 52 air sampler® was connected to the PUF to pull air through the system at a rate of 3 L/min. Air was pulled from the exterior environment through the 0.95 cm hole and across the treated soil flat. Air inside the humidome was then pulled through the PUF to capture dicamba vapors. Humidome design is displayed in Figures 2.3 and Figure 2.4. The experiment was conducted three times.

Treatment design was identical to field experiments. Factor A treatments contained various chloroacetamide herbicides applied at labeled rates. Factor A levels were EC *S*-metolachlor applied as Dual Magnum® at 1.12 kg ai ha<sup>-1</sup>, ME acetochlor applied as Warrant® at 1.27 kg ai ha<sup>-1</sup>, CS pre-mix DGA salt of dicamba + *S*-metolachlor applied as Tavium plus VaporGrip® at 1.68 kg ai ha<sup>-1</sup>, EC acetochlor applied as Harness® at 1.26 kg ai ha<sup>-1</sup>, and no chloroacetamide addition. Factor B levels consisted of potassium salt of glyphosate applied at 0.86 kg ae ha<sup>-1</sup> and no glyphosate addition. Each treatment contained the DGA salt of dicamba with VaporGrip® applied at 0.56 kg ae ha<sup>-1</sup> except for treatments containing the pre-mix of Tavium plus VaporGrip®. Applications were made using an enclosed track sprayer (Series III, Devries Equipment, New Holland, MN) with a carrier volume of 140 L ha<sup>-1</sup>. Soil flats were treated in a separate location and transported to the greenhouse.

Following application, soil flats were placed within their assigned humidomes and sealed using heavy duty duct tape to mitigate vapor escape. Humidomes were then transported to a

greenhouse where attachment of the PUF occurred and air sampling was initiated. Air sampling initiated the start of the 24-hour treatment period.

At the conclusion of the treatment period, PUFs were collected and analyzed by the Mississippi State Chemical Lab using liquid chromatography/ mass spectrometry to provide concentrations of dicamba vapor molecules present in each PUF. Dicamba concentration was quantified using the same methodology described for field PUF analysis. Data were subjected to ANOVA to evaluate significance of main effects and interactions of factors. Data were analyzed using PROC GLIMMIX and means were separated by LSMEANS using an alpha level of 0.05.

## **Results**

No interaction of tank-mixed chloroacetamide herbicide and tank-mixed with glyphosate was detected. Differences among effects within each factor were observed. No site year effects were observed for plant injury, pH, percent of injured plants, and PUF concentration; therefore, data were pooled over site year. Plant height was unaffected.

Injury 14 DAT from treatments containing EC acetochlor expressed increased volatility injury when compared to all other treatments at 49 to roughly 304 cm from treated soil flats, with injury ranging from 20 to below 4% (Figure 2.5). Treatments of the pre-mix of dicamba plus CS *S*-metolachlor showed less dicamba injury than all other treatments at 72 cm and continued to 258 cm from treated flats, with injury from 13% to slightly above 2% (Figure 2.5). Dicamba treatments containing EC *S*-metolachlor, no chloroacetamide, and ME acetochlor expressed no differences in vapor injury with respect to distance (Figure 2.5, Table 2.1). Dicamba vapor injury was observable to 488 cm from the treated soil 14 DAT (Table 2.1). Five percent or greater vapor injury was observed at further distances 14 DAT from treatments containing EC acetochlor (304 cm) when compared to treatments containing ME acetochlor (217 cm) (Figure

2.5). Five percent or greater vapor injury was observed at shorter distances 14 DAT from treatments of the CS premix (168 cm) when compared to treatments containing EC *S*-metolachlor (217 cm) (Figure 2.5).

Averaged over all chloroacetamide treatments, glyphosate increased dicamba phytotoxicity to non-tolerant soybean 14 DAT from distances of 0 to 328 cm when injury was regressed over distance (Figure 2.6). When glyphosate was present, injury ranged from a high of 26% at 0 cm to a low of 3% at 328 cm (Figure 2.6). In the absence of glyphosate, dicamba treatments expressed vapor injury levels below 20% at 0 cm and fell below 1.5% at 328 cm (Figure 2.6) Five percent or greater visual injury was observed to 266 cm with glyphosate present, compared to 164 cm in the absence of glyphosate (Figure 2.6). When each rating distance was analyzed individually averaged over chloroacetamide, glyphosate increased volatility injury at each distance from 0 to 488 cm (Table 2.2).

Dicamba injury regressed over distance did not differ among chloroacetamide additions averaged over glyphosate 28 DAT (Figure 2.7). At 28 DAT, mean dicamba vapor injury greater than 5% was observed out to between 177 and 253 cm from treated soil flat regardless of chloroacetamide addition (Figure 2.7). At 28 DAT, glyphosate increased dicamba vapor injury when averaged over chloroacetamide (Figure 2.8, Table 2.3). Mean dicamba injury resulting from treatments containing glyphosate was greater than non-glyphosate treatments from 0 to 329 cm 28 DAT (Figure 2.8). In the presence of glyphosate, injury ranged from a high of 24% at 0 cm and decreased to a level of 5% at 246 cm 28 DAT (Figures 2.8). Dicamba treatments lacking tank-mixed glyphosate expressed vapor injury levels below 20% at 0 cm and decreased to 5% at 159 cm from the treated soil 28 DAT (Figures 2.8). When each rating point was analyzed

individually averaged over chloroacetamide 28 DAT, glyphosate increased dicamba vapor injury from distances of 0 to 457 cm (Table 2.3).

Under field conditions, dicamba treatments containing EC acetochlor were more volatile than dicamba treatments containing ME acetochlor (Table 2.4). Mean dicamba concentration of PUF samples was 42 ng when an EC formulation of acetochlor was applied, compared to 25 ng from treatments containing ME acetochlor (Table 2.4). Similar formulation effects were observed with *S*-metolachlor. Additions of an EC formulation of *S*-metolachlor resulted in a higher mean dicamba concentration of 42 ng when compared to 27 ng in PUFs from treatments of the CS premix containing *S*-metolachlor (Table 2.4). Treatments lacking chloroacetamide expressed no separation in PUF concentration from any other treatments (Table 2.4). The addition of a chloroacetamide formulated as an EC increased quantifiable volatility when compared to both encapsulated formulations (Table 2.4). In humidome experiments, chloroacetamide addition had no effect on dicamba concentration in PUF samples (Table 2.4). Tank-mixing glyphosate increased PUF concentration from 25 to 42 ng in field experiments (Table 2.4). The effect of tank-mixed glyphosate in humidome experiments agreed with field data by increasing concentration of dicamba in PUFs from 4.37 to 7.29 ng when present in the herbicide solution (Table 2.4).

At 14 DAT, an increase in percentage of injured soybean plants in selected quadrants was observed in treatments containing EC *S*-metolachlor (30%) compared to treatments containing the CS *S*-metolachlor premix (23%) (Table 2.5). Similar observations occurred between formulations of acetochlor 14 DAT. Treatments containing EC formulated acetochlor increased percent of soybean plants injured (37%) when compared to treatments containing ME acetochlor (27%) (Table 2.5). At 28 DAT, no difference in percent injured plants was observed between

chloroacetamide addition by formulation (Table 2.5). Both treatments containing *S*-metolachlor lowered percent of injured soybean plants when compared to EC acetochlor 28 DAT (Table 2.5). The EC acetochlor had an increasing effect on percent injured plants in selected quadrants when compared to treatments without a tank mixed chloroacetamide (Table 2.5). Tank mixing glyphosate increased soybean injury by 9 percent both 14 and 28 DAT (Table 2.5).

Chloroacetamide addition impacted spray solution pH when averaged over levels of glyphosate. Treatments containing ME acetochlor were most alkaline with a mean pH value of 5.17 (Table 2.5). Treatments containing EC *S*-metolachlor and EC acetochlor lacked separation of pH, with mean values of 4.95 and 4.96 respectively (Table 2.5). Treatments lacking tank mixed chloroacetamide had a mean pH value of 4.92 (Table 2.5). The CS *S*-metolachlor and DGA dicamba premix was the most acidic solution with mean pH value of 4.88 (Table 2.5). All chloroacetamide tank mixes increased solution pH except treatments of the CS premix of DGA plus *S*-metolachlor (Table 2.5). Glyphosate had acidifying effects, decreasing mean solution pH from 5.23 to 4.74 when tank mixed (Table 2.5). Glyphosate's acidifying properties on dicamba solution has been observed in previous research (Mueller and Steckel 2019).

These data suggest that chloroacetamide and glyphosate tank-mix decisions can impact severity of dicamba vapor movement. Current label restrictions regarding the tank mixing of *S*-metolachlor and acetochlor support safe application regarding volatility. These tank mixes have little to no effect on volatility when applied with DGA salt of dicamba plus VaporGrip®. Data suggests that the pre-mix containing DGA plus CS *S*-metolachlor is less volatile than mixing *S*-metolachlor and dicamba in the tank. Tank-mixing ME acetochlor with dicamba created less volatility than mixing EC acetochlor. Regardless of tank-mix partner, dicamba volatility was clearly increased when glyphosate was included.

Table 2.1 Effect of chloroacetamide tank mix on dicamba vapor injury of non-DR soybean fourteen days after treatment averaged over glyphosate addition under field conditions<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Chloroacetamide <sup>c</sup>				
	Harness®	Dual Magnum®	No Chloroacetamide %	Warrant®	Tavium®
0	26 a	24 a	22 a	22 a	22 a
30	24 a	22 ab	19 b	20 b	18 b
61	20 a	18 ab	16 ab	14 bc	12 c
91	17 a	12 b	13 ab	11 bc	8 c
122	14 a	11 ab	10 ab	9 b	6 b
152	12 a	9 ab	8 ab	7 b	6 b
183	9 a	7 ab	6 ab	5 b	4 b
213	9 a	5 b	5 b	4 b	3 b
244	8 a	4 b	4 b	4 b	2 b
274	6 a	4 ab	3 b	4 ab	1 b
305	5 a	3 ab	3 bc	3 abc	1 c
335	5 a	3 ab	1 bc	3 ab	0 c
366	4 a	2 bc	1 bc	2 ab	0 c
396	2 a	1 abc	1 bc	1 ab	0 c
427	2 a	1 a	1 a	1 a	0 a
457	1 a	1 a	1 a	1 a	0 a
488	1 a	1 a	0 a	1 a	0 a
518	0 a	0 a	0 a	0 a	0 a
549	0 a	0 a	0 a	0 a	0 a
579	0 a	0 a	0 a	0 a	0 a
610	0 a	0 a	0 a	0 a	0 a
640	0 a	0 a	0 a	0 a	0 a
671	0 a	0 a	0 a	0 a	0 a
701	0 a	0 a	0 a	0 a	0 a
732	0 a	0 a	0 a	0 a	0 a
762	0 a	0 a	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent significance differences between dicamba soybean injury at each individual rating distance represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>Dual Magnum – 2.24 kg ae ha<sup>-1</sup> diglycolamine (DGA) salt of dicamba + 4.4 kg ai ha<sup>-1</sup> *S*-metolachlor as EC formulation; Harness – 2.24 kg a ha<sup>-1</sup> DGA salt of dicamba + 5.04 ka ai ha<sup>-1</sup> of acetochlor as EC formulation; No Chloroacetamide – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba; Tavium – 6.69 kg ai ha<sup>-1</sup> (DGA salt of dicamba + *S*-metolachlor premix) as CS formulation; Warrant – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba + 5.06 kg ai ha<sup>-1</sup> acetochlor as ME formulation

Table 2.2 Effect of glyphosate tank mix on dicamba vapor injury of non-DR soybean fourteen days after treatment averaged over chloroacetamide addition under field conditions<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Glyphosate <sup>c</sup>	
	Glyphosate Tank Mix	No Glyphosate
	----- % -----	-----
0	27 a	20 b
30	23 a	17 b
61	19 a	13 b
91	15 a	9 b
122	13 a	7 b
152	11 a	5 b
183	9 a	4 b
213	7 a	4 b
244	6 a	3 b
274	5 a	2 b
305	4 a	2 b
335	3 a	1 b
366	2 a	1 b
396	2 a	1 b
427	1 a	1 b
457	1 a	0 b
488	1 a	0 b
518	0 a	0 a
549	0 a	0 a
579	0 a	0 a
610	0 a	0 a
640	0 a	0 a
671	0 a	0 a
701	0 a	0 a
732	0 a	0 a
762	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Rounded to nearest percent.

Letters represent differences between dicamba soybean injury at each individual rating distance represented in table row

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>Glyphosate Tank Mix - K-Salt of glyphosate applied at 3.48 kg ae ha<sup>-1</sup> with diglycolamine (DGA) salt of dicamba and various chloroacetamide herbicides; No Glyphosate – no glyphosate mixed with DGA salt of dicamba and various chloroacetamide herbicides



Table 2.3 Effect of glyphosate tank mix on dicamba vapor injury of non-DR soybean twenty-eight days after treatment averaged over chloroacetamide addition under field conditions<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Glyphosate <sup>c</sup>	
	Glyphosate Tank Mix	No Glyphosate
	----- % -----	-----
0	24 a	20 b
30	21 a	17 b
61	17 a	12 b
91	14 a	9 b
122	11 a	7 b
152	9 a	6 b
183	7 a	4 b
213	6 a	3 b
244	6 a	3 b
274	4 a	2 b
305	3 a	1 b
335	2 a	1 b
366	2 a	0 b
396	2 a	1 b
427	1 a	0 b
457	1 a	0 b
488	1 a	0 a
518	0 a	0 a
549	0 a	0 a
579	0 a	0 a
610	0 a	0 a
640	0 a	0 a
671	0 a	0 a
701	0 a	0 a
732	0 a	0 a
762	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Rounded to nearest percent.

Letters represent differences between dicamba soybean injury at each individual rating distance represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>Glyphosate Tank Mix - K-Salt of glyphosate applied at 3.48 kg ae ha<sup>-1</sup> with diglycolamine (DGA) salt of dicamba and various chloroacetamide herbicides; No Glyphosate – no glyphosate mixed with DGA salt of dicamba and various chloroacetamide herbicides

Table 2.4 Effect of tank mixes on dicamba vapor concentration in PUF samples in field and humidome methodology<sup>a</sup>

Factor	Tank Mix <sup>b</sup>	Concentration of Dicamba in PUF <sup>a</sup>	
		Experiment Method	
		Field Conditions <sup>g</sup>	Humidome <sup>h</sup>
<b>Chloroacetamide<sup>cd</sup></b>		----- ng -----	
	Dual Magnum®	42 a	6.09 a
	Harness®	42 a	6.50 a
	No Chloroacetamide	33 ab	5.35 a
	Tavium®	27 b	4.92 a
	Warrant®	25 b	6.32 a
<b>Glyphosate<sup>ef</sup></b>			
	Glyphosate Tank Mix	42 a	7.29 a
	No Glyphosate	25 b	4.37 b

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters show differences of dicamba concentration in columns within factor and experiment method

<sup>b</sup>Tank mixes containing DGA salt of dicamba + VaporGrip™

<sup>c</sup>Effect of chloroacetamide addition averaged over glyphosate effects

<sup>d</sup>Dual Magnum – 2.24 kg ae ha<sup>-1</sup> diglycolamine (DGA) salt of dicamba + 4.4 kg ai ha<sup>-1</sup> *S*-metolachlor as EC formulation; Harness – 2.24 kg a ha<sup>-1</sup> DGA salt of dicamba + 5.04 ka ai ha<sup>-1</sup> of acetochlor as EC formulation; No Chloroacetamide – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba; Tavium – 6.69 kg ai ha<sup>-1</sup> (DGA salt of dicamba + *S*-metolachlor premix) as CS formulation; Warrant – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba + 5.06 kg ai ha<sup>-1</sup> acetochlor as ME formulation

<sup>e</sup>Effect of glyphosate addition averaged over chloroacetamide effects

<sup>f</sup>Glyphosate Tank Mix - K-Salt of glyphosate applied at 3.48 kg ae ha<sup>-1</sup> with diglycolamine (DGA) salt of dicamba and various chloroacetamide herbicides; No Glyphosate – noglyphosate mixed with DGA salt of dicamba and various chloroacetamide herbicides

<sup>g</sup>Herbicides applied at 4x rate using this methodology

<sup>h</sup>Herbicides applied at labeled rate using this methodology

Table 2.5 Effect of tank mixed herbicides on percentage of non-DR soybean injured fourteen and twenty-eight days after treatment and solution pH<sup>a</sup>

Factor	Tank Mix <sup>b</sup>	Percentage of Injured Soybean <sup>g</sup>		pH <sup>h</sup>
		14 DAT	28 DAT	
Chloroacetamide <sup>cd</sup>		----- % -----		
	Harness®	37 a	34 a	4.95 b
	Dual Magnum®	30 ab	25 b	4.96 b
	No Chloroacetamide	27 bc	26 b	4.92 c
	Warrant®	27 bc	29 ab	5.17 a
	Tavium®	23 c	24 b	4.88 d
Glyphosate <sup>ef</sup>				
	Tank Mixed Glyphosate	33 a	32 a	4.74 a
	No Glyphosate	24 b	23 b	5.23 b

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters show differences in percent of injured soybean and solution pH of columns within factor

<sup>b</sup>Tank mixes with DGA salt of dicamba + VaporGrip®

<sup>c</sup>Effect of chloroacetamide addition averaged over glyphosate effects

<sup>d</sup>Dual Magnum – 2.24 kg ae ha<sup>-1</sup> diglycolamine (DGA) salt of dicamba + 4.4 kg ai ha<sup>-1</sup> *S*-metolachlor as EC formulation; Harness – 2.24 kg a ha<sup>-1</sup> DGA salt of dicamba + 5.04 ka ai ha<sup>-1</sup> of acetochlor as EC formulation; No Chloroacetamide – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba; Tavium – 6.69 kg ai ha<sup>-1</sup> (DGA salt of dicamba + *S*-metolachlor premix) as CS formulation; Warrant – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba + 5.06 kg ai ha<sup>-1</sup> acetochlor as ME formulation

<sup>e</sup>Effect of glyphosate addition averaged over chloroacetamide effects

<sup>f</sup>Glyphosate Tank Mix - K-Salt of glyphosate applied at 3.48 kg ae ha<sup>-1</sup> with diglycolamine (DGA) salt of dicamba and various chloroacetamide herbicides; No Glyphosate – no glyphosate mixed with DGA salt of dicamba and various chloroacetamide herbicides

<sup>g</sup>Percentage of injured soybean plants within selected quadrants in field experiments

<sup>h</sup>Solution pH measurements following application at room temperature

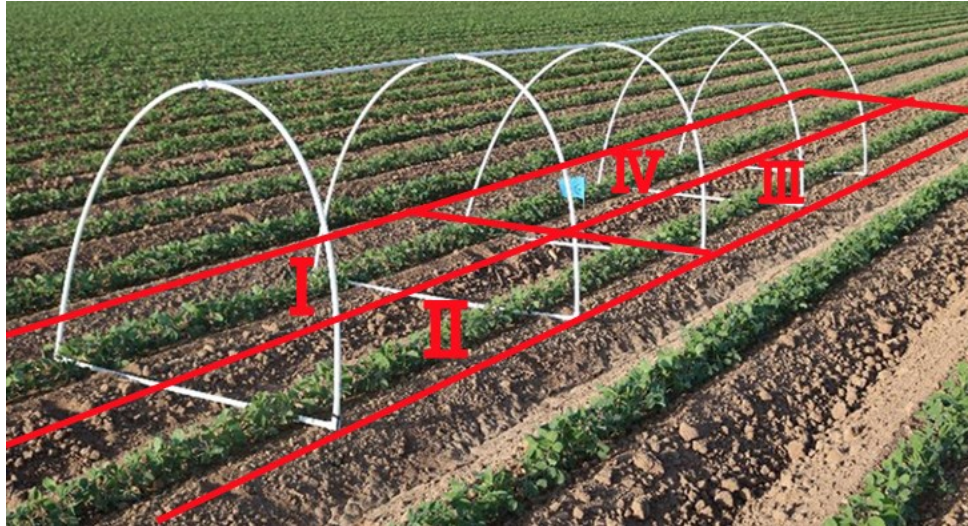


Figure 2.1 PVC frame of low-tunnel tent with quadrant diagram



Figure 2.2 Completed low-tunnel tent frame with contractor's plastic covering

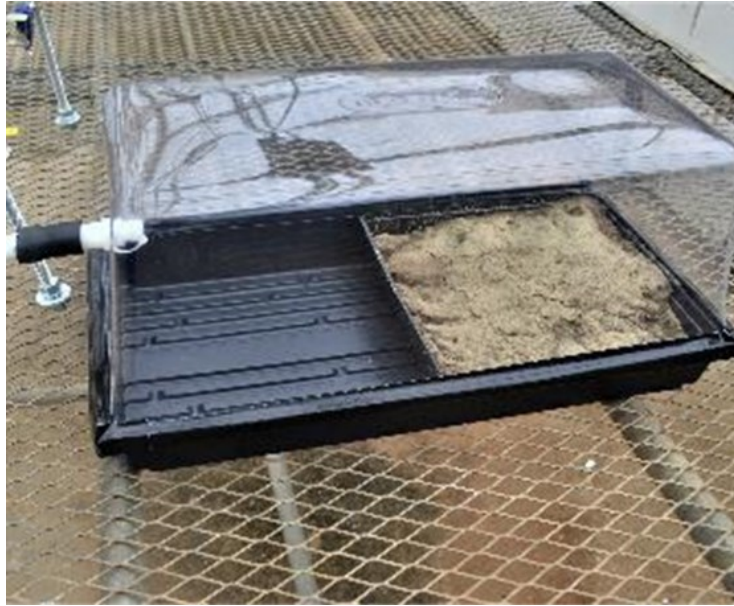


Figure 2.3 Humidome design with 1010 soil flat and sealed humidity dome



Figure 2.4 Humidome experiment design

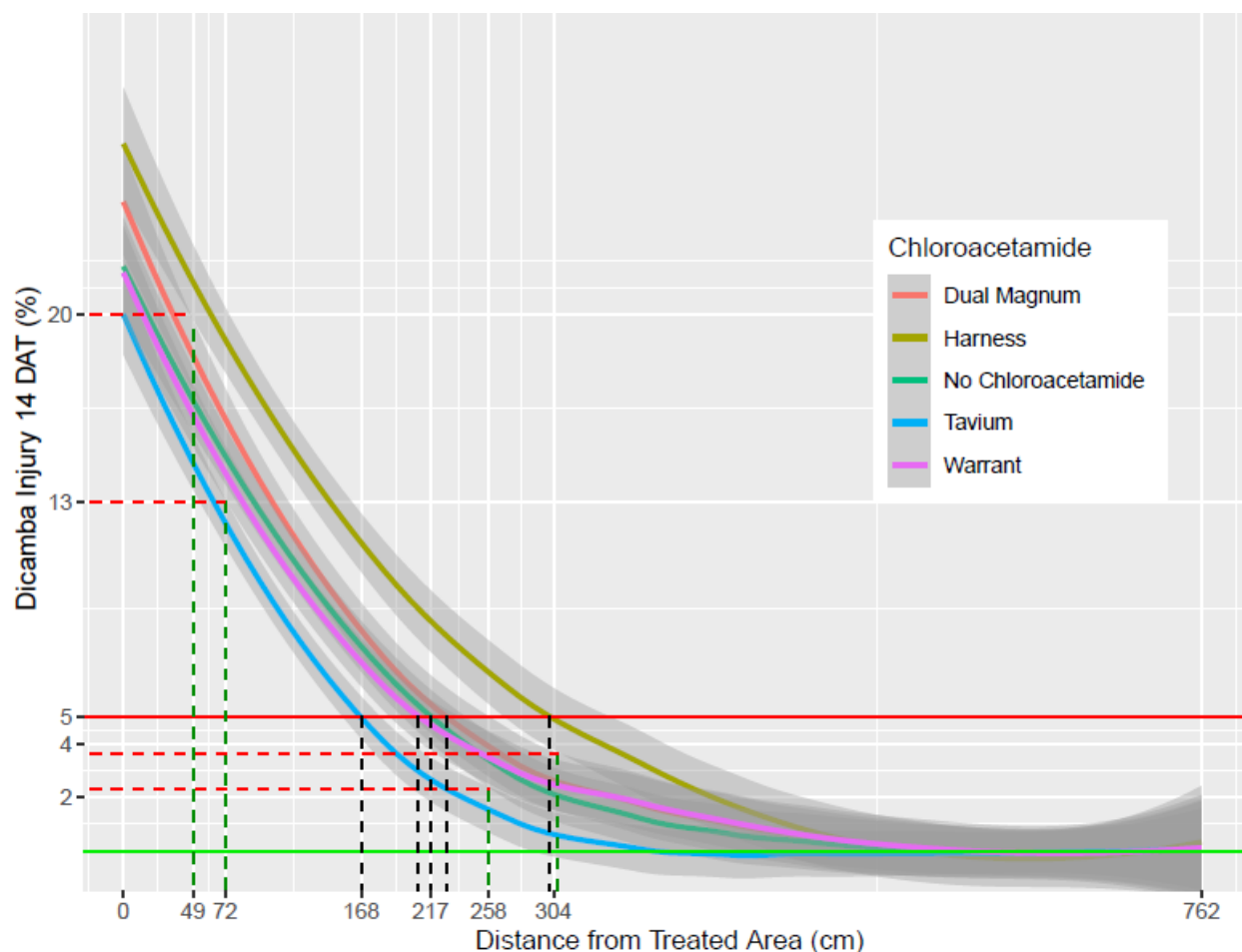


Figure 2.5 Effect of chloroacetamide averaged over glyphosate on dicamba vapor injury of non-DR soybean regressed over distance fourteen days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>Dual Magnum – 2.24 kg ae ha<sup>-1</sup> diglycolamine (DGA) salt of dicamba + 4.4 kg ai ha<sup>-1</sup> *S*-metolachlor in EC formulation; Harness – 2.24 kg a ha<sup>-1</sup> DGA salt of dicamba + 5.04 ka ai ha<sup>-1</sup> in EC formulation; No Chloroacetamide – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba; Tavium plus VaporGrip – 6.69 kg ai ha<sup>-1</sup> (DGA salt of dicamba + *S*-metolachlor premix) in CS formulation; Warrant – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba + 5.06 kg ai ha<sup>-1</sup> acetochlor in ME formulation



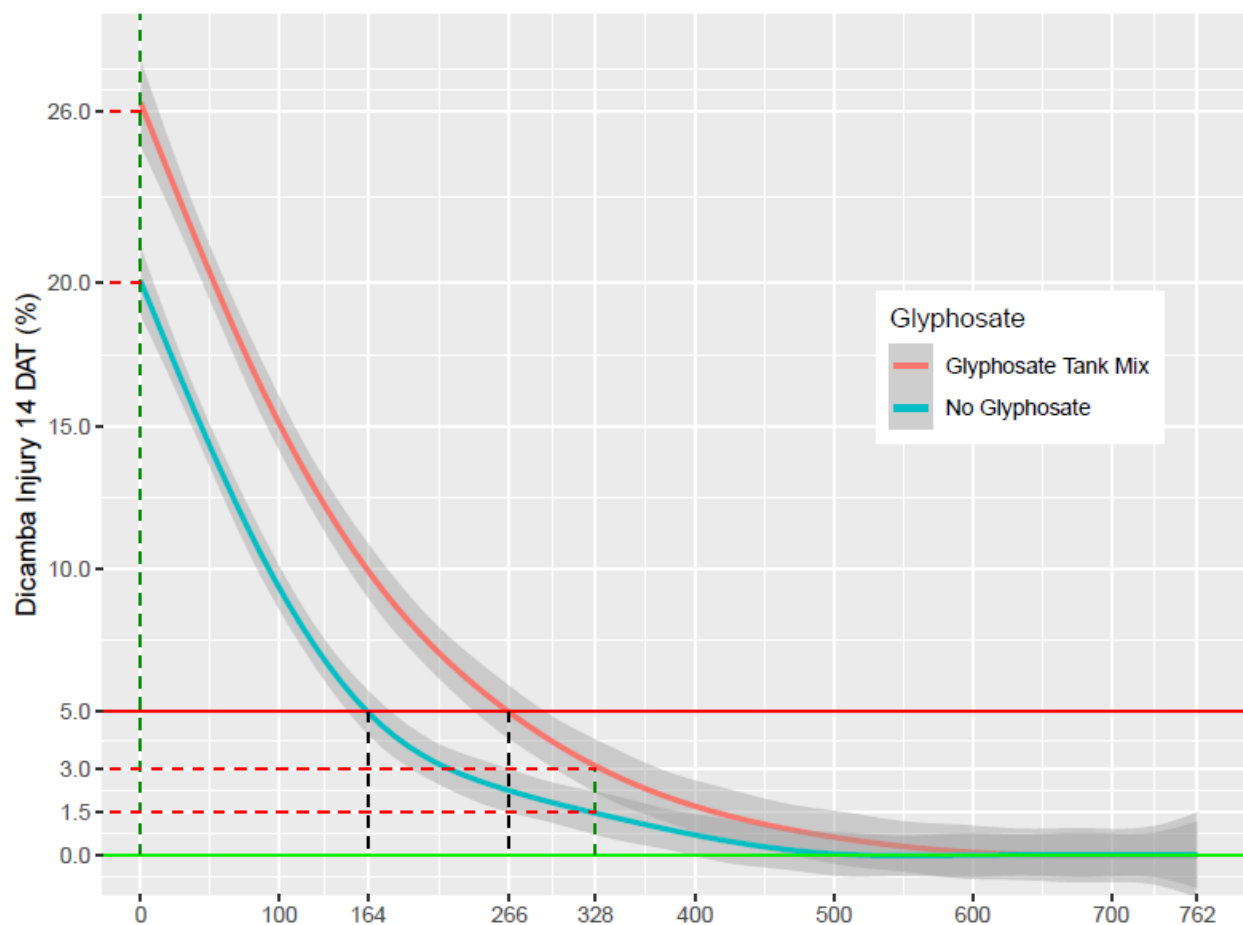


Figure 2.6 Effect of glyphosate averaged over chloroacetamide on dicamba vapor injury of non-DR soybean regressed over distance fourteen days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>K salt of glyphosate tank mixed at rate of 3.47 kg ae ha<sup>-1</sup> with DGA salt of dicamba and various chloroacetamides

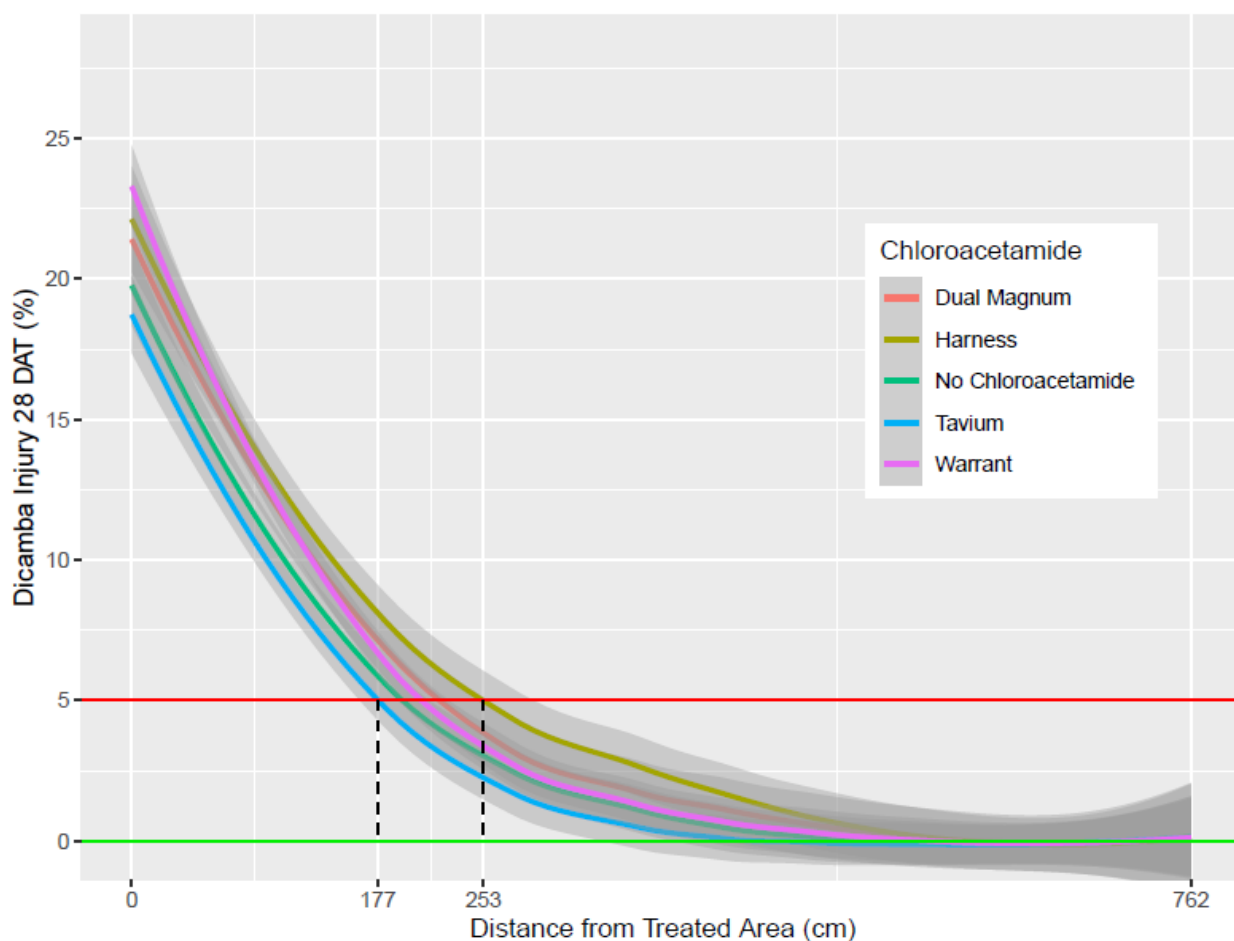


Figure 2.7 Effect of chloroacetamide averaged over glyphosate on dicamba vapor injury of non-DR soybean regressed over distance twenty-eight days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Green line represents no injury. Red line represents 5% injury observation; Vertical black lines represent mean distances of 5% injury observation by tank mix

<sup>c</sup>Dual Magnum – 2.24 kg ae ha<sup>-1</sup> diglycolamine (DGA) salt of dicamba + 4.4 kg ai ha<sup>-1</sup> *S*-metolachlor as EC formulation; Harness – 2.24 kg a ha<sup>-1</sup> DGA salt of dicamba + 5.04 ka ai ha<sup>-1</sup> as EC formulation; No Chloroacetamide – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba; Tavium – 6.69 kg ai ha<sup>-1</sup> (DGA salt of dicamba + *S*-metolachlor premix) as CS formulation; Warrant – 2.24 kg ae ha<sup>-1</sup> DGA salt of dicamba + 5.06 kg ai ha<sup>-1</sup> acetochlor as ME formulation



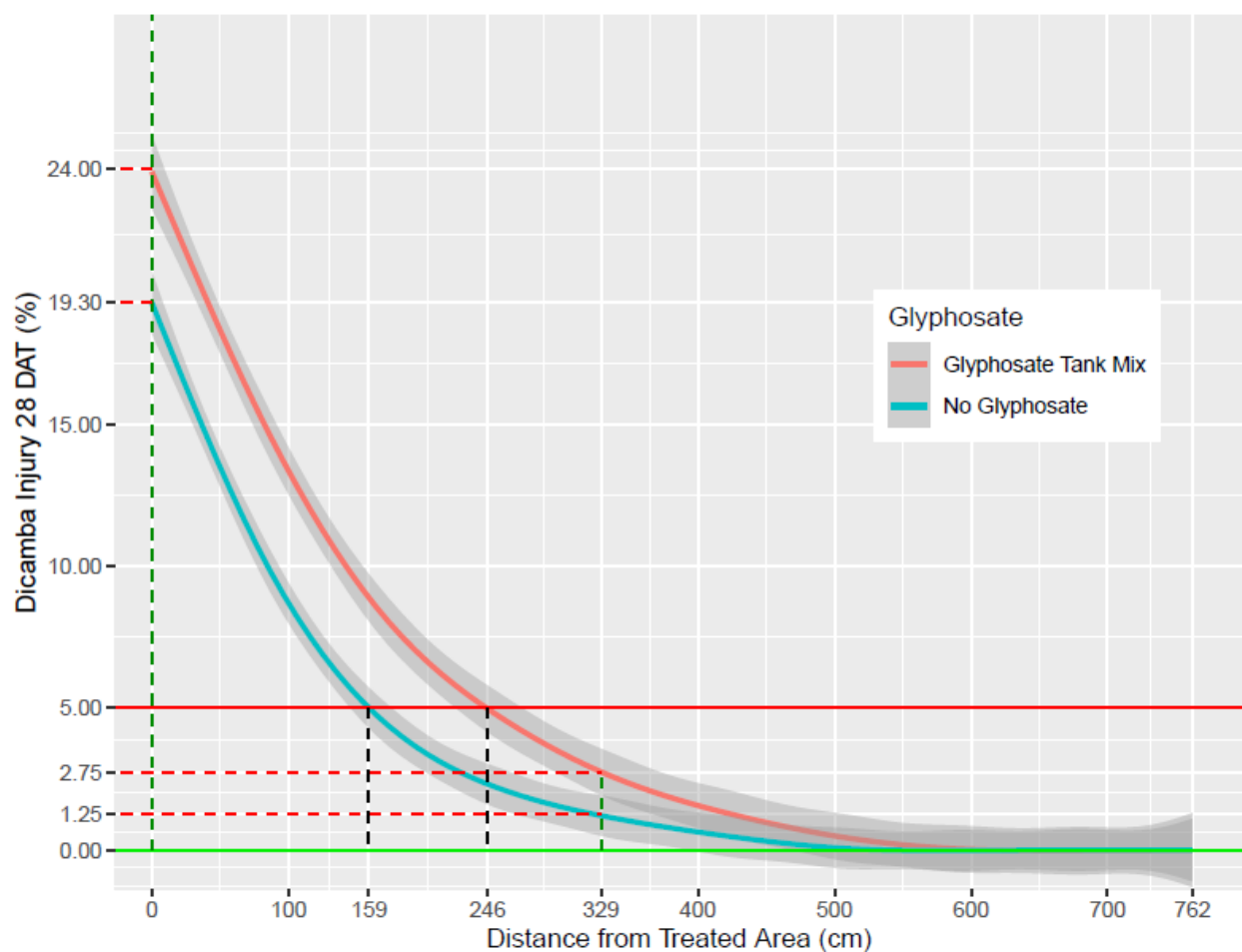


Figure 2.8 Effect of glyphosate averaged over chloroacetamide on dicamba vapor injury of non-DR soybean regressed over distance twenty-eight days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>Glyphosate tank mixed at rate of 3.47 kg ae ha<sup>-1</sup> with DGA salt of dicamba and various chloroacetamides

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## CHAPTER III

### EFFECT OF IRRIGATION ON VAPOR MOVEMENT OF DICAMBA

#### **Abstract**

Dicamba off-target-movement (OTM) has become more common due to its increased application frequency following introduction of dicamba resistant (DR) crops. One form of dicamba OTM is volatility. Dicamba volatility is influenced by weather conditions, including air temperature, relative humidity, and rainfall. Rainfall following application has been shown to decrease dicamba volatility. Manipulation of rainfall through irrigation could provide additional farm-level mitigation efforts of dicamba volatility. Field experiments were conducted in 2019 and 2020 to evaluate mitigation efforts of irrigation following a commonly applied dicamba tank mix. Herbicide applications consisted of dicamba with VaporGrip™ applied at 2.24 kg ae ha<sup>-1</sup> in tank-mix with the potassium salt of glyphosate applied at 4.49 kg ae ha<sup>-1</sup>. Herbicides were applied at 4X rates to ensure visual symptomology. Irrigation quantities of 0, 1.6, 3.2, 6.4, 9.6, and 12.7 mm were applied following dicamba application. Both herbicide and irrigation were applied to greenhouse flats filled with field soil. Following treatment and simulated irrigation, treated flats were placed between two rows of soybean. Plots measured 15.3 m in length and contained a 6.2 x 1.5 m low-tunnel tent covered with plastic. At 14 DAT, the non-irrigated treatment showed more injury than those receiving any quantity of irrigation. The 12.7 mm irrigation treatment resulted in no crop injury. At 28 DAT, all irrigated treatments reduced

volatility and crop injury. Treatments receiving 6.4 to 12.7 mm of irrigation exhibited less injury than those receiving 0 to 3.2 mm. Dicamba concentration in the non-irrigated treatment was 43 ng compared to the 1 to 5 ng found for all irrigation treatments. Injury and dicamba concentrations suggest irrigation following herbicide application mitigates volatility. Increasing amounts of irrigation following application has positive impacts on mitigation of dicamba volatility.

**Nomenclature:**

Diglycolamine salt of dicamba; potassium salt of glyphosate; soybean, *Glycine max* (L.) Merr.

**Key Words:**

Irrigation; rainfall; volatility

**Introduction**

Dicamba-resistant (DR) cropping technology introduced dicamba tolerance into soybean and *Gossypium hirsutum* L. production. The technology enabled POST application of dicamba to control problematic broadleaf weed species and provided an additional option for growers struggling with control of glyphosate resistant weeds (Behrens et al. 2007). Adoption of DR soybean occurred rapidly after introduction in 2016 (Wechsler et al. 2019). Between 2016 and 2018, DR soybean hectares increased 43% in the U.S. (Wechsler et al. 2019). In the 2018 Mississippi cropping season, 79% of the soybean hectares were planted with DR soybean (Wechsler et. al 2019). Rapid adoption and expansion of dicamba use in DR crops has created concern for undesired mobility of the auxin into regions of sensitive plant production.

Dicamba, a synthetic auxin belonging to the benzoic acid family of herbicides (Bunch et al. 2012), provides control of broadleaf weed species by mimicking hormonal properties of endogenous auxins present in plants (Grossmann 2010, Sterling and Hall 1997). Upon uptake, unnaturally high levels of auxins present in plant tissue cause symptomatic response at low rates and death at higher rates (Grossmann 2010, Bunch et al. 2012, Sterling and Hall 1997).

Phytotoxic response occurs as epinastic growth, leaf curling, node stacking, and other forms of unnatural growth following exposure (Behrens and Lueschen 1979, Sterling and Hall 1997).

Symptomatic response of sensitive plants can occur at exposure rates far below labeled use rates (Hartzler 2017). For sensitivity comparison, glyphosate use rates of 1% of labeled rates cause visible injury to susceptible *Zea mays* L., while only 0.005% of labeled use rates of dicamba are required to cause injury in susceptible soybean cultivars (Hartzler 2017).

Sensitivity of susceptible crops and plants has contributed to the observance of dicamba off-target movement (OTM) injury (Hartzler 2017). Dicamba OTM occurs as physical drift, tank contamination, and vapor movement (Soltani et al. 2020, Behrens and Lueschen 1979, Boerboom 2004). These forms of OTM often occur at rates far below labeled use rates but provide adequate dicamba concentrations for the development of symptomology in sensitive plants (Carlsen et al. 2006; Egan et al. 2014). Physical drift is the movement of herbicide solution at the time of application by wind (Soltani et al. 2020). Tank contamination results from application of residual dicamba molecules present in the spray tank, hoses, and pumps of spray equipment following proper cleanout procedure (Boerboom 2004). Vapor movement, or volatility, is the movement of pesticide vapors as a gas or fumes by wind (Maybank et al. 1978, Grover 1975). Vapor movement occurs after application, resulting from transition of liquid herbicide solution or solids into a gaseous state (Behrens and Lueschen 1979). The release and

adoption of DT crop technology resulted in increased use of dicamba and expanded potential sources of OTM.

Expanded use of auxin herbicides has increased efforts in regulatory action to mitigate OTM and ease tracking of sources (EPA 2020). Restriction of dicamba formulation, limiting of application timing, restriction of legal tank mixtures, droplet size requirements, required applicator training, requiring use of drift reducing agents (DRA), and volatility reduction agents (VRA) have been implemented to mitigate dicamba OTM (Mississippi State University Extension, Anonymous 2020a, Anonymous 2020b, Anonymous 2018). Specific application records are also required to ease source tracking of dicamba OTM and ensure applicators understand environmental conditions and factors when applying dicamba (Mississippi State University Extension). Despite numerous regulatory actions and mitigation efforts, many hectares of non-tolerant soybean have expressed dicamba symptomology resulting from OTM (Bradley 2017, Hager 2017, Steckel et al. 2017).

Successful mitigation of dicamba OTM requires specific attention to each application required by dicamba label (Anonymous 2018, 2020a, 2020b). Physical drift mitigation efforts utilize low-drift nozzle design, use of drift reduction agents, and monitoring of current weather conditions (Foster et al. 2018, Creech et al. 2015, Womac et al. 1997). Tank contamination mitigation utilizes segregation of spray equipment, proper cleanout procedure, or use of sprayer components less likely to sequester dicamba molecules (Cundiff et al. 2017). Vapor movement mitigation efforts include use of a VRA, monitoring weather conditions, timing of application, and understanding of tank mix effects (Behrens and Lueschen 1979, Mueller et al. 2013, EPA 2020). Weather variability following application creates difficulty in mitigating dicamba vapor movement contributing to OTM injury.



The inability to control weather conditions following dicamba application creates uncertainty in application fate (Mueller et al. 2013, Behrens and Lueschen 1979). Following application, dicamba aerosols can remain suspended and allow for movement up to 48-hours (Mueller et al. 2013). This time of vapor formation can broaden volatility concern with changing weather conditions. Changes in wind speed, air temperature, relative humidity, and rainfall during this post-application volatility window have been found to impact severity and distance of dicamba vapor movement (Behrens and Lueschen 1979, Hartzler 2017). Increasing wind speed, air temperature, and relative humidity create volatility promoting conditions, while rainfall events following dicamba application are found to decrease the extent of dicamba vapor movement and injury of non-tolerant crops (Bauerle et al. 2015, Behrens and Lueschen 1979, Hartzler 2017).

Rainfall events result in downward movement of dicamba into the soil profile (Hall and Mumma 1994, Grover 1977). Due to its anionic behavior, dicamba is prone to downward leaching through soil profiles with dissipation occurring within 8-14 days (Hall and Mumma 1994). Compared to other auxin herbicides, dicamba requires less precipitation to reach 10 cm of downward movement from overhead irrigation or rainfall (Grover 1977). Dicamba present in the soil solution is unable to volatilize and has previously been found less likely to result in vapor movement injury (Behrens and Lueschen 1979, Bauerle et al. 2015).

Use of irrigation has continually expanded worldwide, with annual growth rates between 1 to 4.1% since 1950 (Jensen et al. 1990). Methods of irrigation commonly include surface and overhead sprinkler irrigation, with regional specific irrigation methods varying with crop characteristics and water availability (Bjorneberg 2013). In Mississippi, roughly 60% of cropland is irrigated by furrow and center-pivot overhead irrigation (Kebede et al. 2014). Utilizing

expanding irrigation capabilities, applicators could improve mitigation efforts of dicamba volatility using post-applied irrigation. Research will investigate the utility of irrigation as a mitigating factor following dicamba application and the amount of irrigation required to mitigate substantial dicamba volatility with modern dicamba formulations and tank mixtures.

## **Materials and Methods**

In 2019 and 2020, experiments were conducted in Starkville, MS, at the R.R. Foil Experiment Station to evaluate the effects of various amounts of irrigation on dicamba volatility. Experiments were conducted using a randomized complete block design with three replications. A total of three experiments were conducted. Treatments consisted of identical herbicide applications followed by various irrigation amounts. Herbicide treatments contained the diglycolamine salt of dicamba (DGA) + VaporGrip™ at 2.24 kg ae ha<sup>-1</sup> tank-mixed with a K salt of glyphosate at 4.49 kg ae ha<sup>-1</sup>. Treatments were applied with a carrier volume of 140 L ha<sup>-1</sup>. Herbicide applications used 4X labeled rates applied in a single spray boom pass to ensure phytotoxic response of indicator soybean. Irrigation quantities of 0, 1.6, 3.2, 6.4, 9.6, and 12.7 mm were applied using Wilger DR110-10 Combo-Jet nozzles (Wilger Inc., Lexington, TN) at 0.19 kmph to simulate overhead irrigation following herbicide application. Irrigation calibration occurred by individual irrigation boom pass, with increasing irrigation amount applied by increasing number of passes. Herbicide and irrigation application were done using a DeVries Series III research track sprayer (Devries Equipment, New Holland, MN). Treatments were applied to soil-filled greenhouse flats (Heavy Duty 1020 Tray, Greenhouse Megastore) kept weed free and uniform in surface texture. Soil flats were transported to the experiment site via truck bed following herbicide and irrigation application.

Plots measured 15.3 x 0.8 m, with replications separated by an alley measuring 6.1 m. Two unplanted rows measuring 2.3 m in width separated plots within the same replication to mitigate potential vapor movement between plots. Two rows of ‘Terral 51A56’ soybean were planted at a seeding rate of 345,940 seeds ha<sup>-1</sup> to act as indicator plants within plots. Treatment application occurred between V4 and V5 vegetative soybean growth stages to ensure dicamba exposure prior to initiation of reproductive structures. A 1.5 x 4.6 m PVC frame was placed in the center of each plot and treated soil flats placed beneath (Figure 3.1). Following flat transportation and placement within plots, contractor’s plastic was draped over the PVC structure and clamped on both ends (Figure 3.2). Ends of the low tunnel remained open to allow air movement through the tunnel. Treated greenhouse flats and low tunnels remained in each plot for a 48-hour exposure period. Following the 48-hour exposure period, all low tunnels, contractor’s plastic, and soil flats were removed from the field.

Collection of visible injury and plant heights occurred 14 and 28 days after treatment (DAT). Evaluations began from the conclusion of the 48-hour exposure period. One to two days prior to the first evaluation, the most injured quadrant of each plot was identified, and evaluations occurred within this quadrant at both timings (Figure 3.1). Evaluations in the selected quadrant occurred outward from the center of the plot in 30 cm intervals. Plant injury ratings used a percentage scale from 0 to 100% compared to the untreated check (Behrens and Lueschen 1979). Plant heights were taken in centimeters.

At the center of each plot, placed 30.5 cm above treated soil flats, a low-volume polyurethane foam tube (PUF) (SKC, Eighty-Four, PA) collected dicamba molecules volatilizing from treated soil flats. Each PUF was connected to an SKC AirChek 52 (SKC, Eighty-Four, PA) air sampler calibrated to pull air through the PUF at a rate of 3 Liters per minute. Air sampling

occurred for the entirety of the 48-hour exposure period, with PUFs collected at its conclusion. Individual PUFs were analyzed by the Mississippi State Chemical Lab using liquid chromatography/mass spectrometry to analyze concentrations of dicamba molecules in nanograms(ng).

Dicamba concentration was quantitated using an Agilent 1290 liquid chromatograph (Agilent Technologies, Santa Clara, CA) coupled with an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed using an Agilent Zorbax Eclipse Plus 100 mm column. The mobile phases consisted of 0.1% formic acid in water for the aqueous phase (A) and 0.1% formic acid in acetonitrile as the organic phase. The flow rate 0.3 mL per minute with the following gradient program: 0 to 0.5 min of 25% B, 0.5 to 1 min of 50% B, and 1 to 4 min of 60% B. The ionization of dicamba was preformed using electrospray ionization (ESI) in negative mode with an auxiliary gas ( $N^2$ ), source temperature of 200°C, and a gas flow rate of 10 Liters per minute.

Data analysis used SAS 9.4 (SAS Institute Inc, Cary, NC) and RStudio (RStudio Inc, Boston, MA). Data were subjected to ANOVA to evaluate significance and interactions of factors. Data were analyzed using PROC GLIMMIX and means separated by LSMEANS with an alpha level of 0.05 in SAS 9.4. Plant injury and plant height data were also non-linearly regressed with a 95% confidence band using the loess package in RStudio due to non-parametric behavior of the data (Scholtes et al. 2019).

## **Results**

No differences were found between site-year, so data were pooled over site-year. Differences in dicamba volatility and injury were observed among treatments. Plant heights were unaffected.

At 0 cm 14 DAT, no separation was found between the treatment receiving no irrigation and those receiving 1.6 mm (Table 3.1). At 0 cm 14 DAT, treatments receiving 3.2, 6.4, and 9.6 mm expressed less vapor injury than treatments receiving no irrigation (Table 3.1). Beginning at 0 cm 14 DAT, the treatment receiving 12.7 mm of irrigation lacked injury separation from the non-treated check (Table 3.1). Beginning at 30 cm 14 DAT, all irrigated treatments produced less injury than the treatment receiving no irrigation (Table 3.1). Beginning at 61 cm 14 DAT, the treatment receiving 9.6 mm of irrigation lacked separation of vapor injury from the non-treated check (Table 3.1). Beginning at 91 cm 14 DAT, no difference in vapor injury was observed between any irrigated treatment and the non-treated check (Table 3.1).

Vapor injury regressed over distance found treatments receiving no irrigation expressed more injury than all irrigated treatments from 0 to 279 cm 14 DAT, with injury ranging from 26.0 to greater than 0.5% (Figure 3.3). Treatments receiving 12.7 mm of irrigation were injured least between distances of 0 to 111 cm, with injury ranging from 7 to under 3% (Figure 3.3). Treatments receiving 1.6, 3.2, 6.4, and 9.6 mm lacked vapor injury separation at any distance 14 DAT but were all less injurious than treatments receiving no irrigation from 0 to 279 cm (Figure 3.3)

Treatments receiving no irrigation after application caused vapor injury of 5% or greater at further distances than all other treatments 14 DAT (Figure 3.3). Treatments receiving no irrigation caused injury of 5% or more to 200 cm 14 DAT (Figure 3.3). Treatments receiving 1.6, 3.2, 6.4, and 9.6 mm had no separation 14 DAT, causing injury of 5% or more within a range of 69 to slightly above 111 cm (Figure 3.3). The treatment receiving 12.7 mm of irrigation caused injury of 5% or greater to only 35 cm (Figure 3.3).

At 0 cm 28 DAT, no separation in vapor injury was observed between treatments receiving 0 to 3.2 mm of irrigation (Table 3.2). At 0 cm 28 DAT, treatments receiving 6.4 to 12.7 mm of irrigation expressed less vapor injury than treatments receiving 0 to 3.2 mm (Table 3.2). Beginning at 0 cm 28 DAT, vapor injury from treatments receiving 12.7 mm of irrigation did not differ from the non-treated check (Table 3.2). Beginning at 61 cm and continuing through 427 cm 28 DAT, all irrigated treatments expressed less vapor injury than the treatment receiving no irrigation (Table 3.2). Beginning at 152 cm 28 DAT, no difference in vapor injury was found between the non-treated check and any irrigated treatments (Table 3.2)

The treatment receiving no irrigation resulted in more severe vapor injury than all other treatments from 0 to 363 cm when injury was regressed over distance 28 DAT (Figure 3.4). Injury within this range spanned from 26 to greater than 1% (Figure 3.4). Treatments receiving 12.7 mm of irrigation expressed less injury than all other treatments from 0 to 102 cm 28 DAT, with injury ranging from nearly 9 to below 4% (Figure 3.4). Treatments receiving 1.6, 3.2, 6.4, and 9.6 mm had no separation in vapor injury response when non-linearly regressed over distance 28 DAT but produced less vapor injury than treatments receiving no irrigation from 0 to 363 cm (Figure 3.4).

By 28 DAT, treatments receiving no irrigation resulted in 5% injury or greater to 250 cm from treated soil flats (Figure 3.4). In comparison, treatments receiving 12.7 mm of irrigation expressed 5% or greater vapor injury to only 58 cm (Figure 3.4). Dicamba treatments receiving 1.6 – 9.6 mm of irrigation lacked separation in distance of 5% vapor injury or greater 28 DAT, with observation within a range of 80 to 130 cm (Figure 3.4).

Percentage of injured plants was calculated within the selected plot quadrant 14 and 28 DAT. All treatments receiving any irrigation produced a lower percentage of injured soybean

plants than treatments receiving no irrigation at both rating timings (Table 3.3). The treatment receiving 12.7 mm of irrigation lacked separation of percent injured plants from the non-treated check at both rating timings (Table 3.3). Treatments receiving 1.6 to 9.6 mm of irrigation produced a higher percentage of injured plants than the non-treated check at both rating timings (Table 3.3).

The treatment receiving no irrigation produced higher dicamba vapor concentrations than all other treatments, expressing a PUF concentration of 43 ng (Table 3.3). No separation was observed among all irrigated treatments, producing quantifiable dicamba concentrations of 1 to 5 ng in PUFs (Table 3.3). All irrigated treatments lacked separation of PUF concentration from the non-treated check (Table 3.3).

These data suggest that irrigation applied following dicamba application mitigates vapor injury potential of a dicamba application. Results suggest that all levels of irrigation mitigate and reduce severity of dicamba volatility, but mitigation success improves as irrigation amount is increased. Due to mitigation ability, the use of irrigation following herbicide application could assist growers in mitigation efforts at the farm-level where irrigation is applicable and applied following the rainfast interval. Use of irrigation following dicamba application could also provide registration agencies and agrochemical companies with additional mitigation regulation alongside continuous OTM concern and potentially limit cases of injurious vapor movement.

Table 3.1 Effect of simulated irrigation quantity on dicamba phytotoxic response of non-DR soybean by evaluation distance fourteen days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Irrigation Following Herbicide Application (mm) <sup>c</sup>						Nontreated
	0	1.6	3.2	6.4	9.6	12.7	
	----- % -----						
0	26 a	18 ab	15 bc	12 bc	15 bc	7 cd	0 d
30	22 a	13 b	11 b	10 b	11 b	7 bc	0 c
61	19 a	7 b	8 b	7 b	3 bc	4 bc	0 c
91	14 a	3 b	4 b	3 b	1 b	1 b	0 b
122	11 a	2 b	2 b	1 b	0 b	0 b	0 b
152	6 a	1 b	1 b	1 b	0 b	0 b	0 b
183	5 a	0 b	1 b	0 b	0 b	0 b	0 b
213	3 a	0 b	0 b	0 b	0 b	0 b	0 b
244	2 a	0 b	0 b	0 b	0 b	0 b	0 b
274	2 a	0 b	0 b	0 b	0 b	0 b	0 b
305	2 a	0 b	0 b	0 b	0 b	0 b	0 b
335	2 a	0 b	0 b	0 b	0 b	0 b	0 b
366	1 a	0 b	0 b	0 b	0 b	0 b	0 b
396	0 a	0 a	0 a	0 a	0 a	0 a	0 a
427	0 a	0 a	0 a	0 a	0 a	0 a	0 a
457	0 a	0 a	0 a	0 a	0 a	0 a	0 a
488	0 a	0 a	0 a	0 a	0 a	0 a	0 a
518	0 a	0 a	0 a	0 a	0 a	0 a	0 a
549	0 a	0 a	0 a	0 a	0 a	0 a	0 a
579	0 a	0 a	0 a	0 a	0 a	0 a	0 a
610	0 a	0 a	0 a	0 a	0 a	0 a	0 a
640	0 a	0 a	0 a	0 a	0 a	0 a	0 a
671	0 a	0 a	0 a	0 a	0 a	0 a	0 a
701	0 a	0 a	0 a	0 a	0 a	0 a	0 a
732	0 a	0 a	0 a	0 a	0 a	0 a	0 a
762	0 a	0 a	0 a	0 a	0 a	0 a	0 a

<sup>a</sup> Means are averaged over locations separated by LSMEANS (alpha = 0.05). Letters represent mean separation of dicamba injury between treatment at each individual rating distance represented in table rows.

<sup>b</sup> Distances rounded to nearest cm

<sup>c</sup> Application of diglycolamine salt of dicamba at 2.24 kg ae ha<sup>-1</sup> + potassium salt of glyphosate at 4.49 kg ae ha<sup>-1</sup> with a carrier volume of 140 L ha<sup>-1</sup> followed by overhead irrigation



Table 3.2 Effect of simulated irrigation quantity on dicamba phytotoxic response of non-DR soybean by evaluation distance twenty-eight days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Irrigation Following Herbicide Application (mm) <sup>c</sup>						Nontreated
	0	1.6	3.2	6.4	9.6	12.7	
	----- % -----						
	--						
0	26 a	18 ab	19 ab	14 bc	16 bc	9 cd	0 d
30	21 a	11 ab	12 ab	11 b	11 b	8 bc	0 c
61	20 a	6 bc	10 b	9 b	6 bc	6 bc	0 c
91	19 a	3 bc	6 b	4 b	2 bc	2 bc	0 c
122	13 a	2 bc	3 b	1 bc	1 bc	0 c	0 c
152	11 a	1 b	1 b	0 b	0 b	0 b	0 c
183	7 a	0 b	0 b	0 b	0 b	0 b	0 b
213	5 a	0 b	0 b	0 b	0 b	0 b	0 b
244	4 a	0 b	0 b	0 b	0 b	0 b	0 b
274	4 a	0 b	0 b	0 b	0 b	0 b	0 b
305	3 a	0 b	0 b	0 b	0 b	0 b	0 b
335	3 a	0 b	0 b	0 b	0 b	0 b	0 b
366	3 a	0 b	0 b	0 b	0 b	0 b	0 b
396	2 a	0 b	0 b	0 b	0 b	0 b	0 b
427	1 a	0 b	0 b	0 b	0 b	0 b	0 b
457	0 a	0 a	0 a	0 a	0 a	0 a	0 a
488	0 a	0 a	0 a	0 a	0 a	0 a	0 a
518	0 a	0 a	0 a	0 a	0 a	0 a	0 a
549	0 a	0 a	0 a	0 a	0 a	0 a	0 a
579	0 a	0 a	0 a	0 a	0 a	0 a	0 a
610	0 a	0 a	0 a	0 a	0 a	0 a	0 a
640	0 a	0 a	0 a	0 a	0 a	0 a	0 a
671	0 a	0 a	0 a	0 a	0 a	0 a	0 a
701	0 a	0 a	0 a	0 a	0 a	0 a	0 a
732	0 a	0 a	0 a	0 a	0 a	0 a	0 a
762	0 a	0 a	0 a	0 a	0 a	0 a	0 a

<sup>a</sup>Means are averaged over locations separated by LSMEANS ( $\alpha = 0.05$ ). Letters represent mean separation of dicamba injury between treatment at each individual rating distance represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>Application of diglycolamine salt of dicamba at  $2.24 \text{ kg ae ha}^{-1}$  + potassium salt of glyphosate at  $4.49 \text{ kg ae ha}^{-1}$  with a carrier volume of  $140 \text{ L ha}^{-1}$  followed by overhead irrigation

Table 3.3 Effect of simulated irrigation quantity on percentage of dicamba injured soybean and quantifiable dicamba volatility<sup>a</sup>

Irrigation Following Herbicide Application <sup>b</sup> (mm)	Factor		
	Percentage of Injured Soybean <sup>c</sup>		Dicamba Concentration in PUF <sup>d</sup>
	14 DAT	28 DAT	
	----- % -----	-----	----- ng -----
0	32 a	27 a	43 a
1.6	13 b	8 b	5 b
3.2	12 cb	8 b	3 b
6.4	10 cb	7 b	2 b
9.6	8 cb	6 b	1 b
12.7	7 cd	3 cb	1 b
Nontreated	0 d	0 c	0 b

<sup>a</sup> Means are averaged over locations separated by LSMEANS (alpha = 0.05). Letters represent separation of means within column

<sup>b</sup> Application of diglycolamine salt of dicamba at 2.24 kg ae ha<sup>-1</sup> + potassium salt of glyphosate at 4.49 kg ae ha<sup>-1</sup> with a carrier volume of 140 L ha<sup>-1</sup> followed by overhead irrigation

<sup>c</sup> Calculated in selected quadrant as percentage of injured soybean plants in quadrant stand count

<sup>d</sup> Quantified using liquid chromatography/mass spectrometry

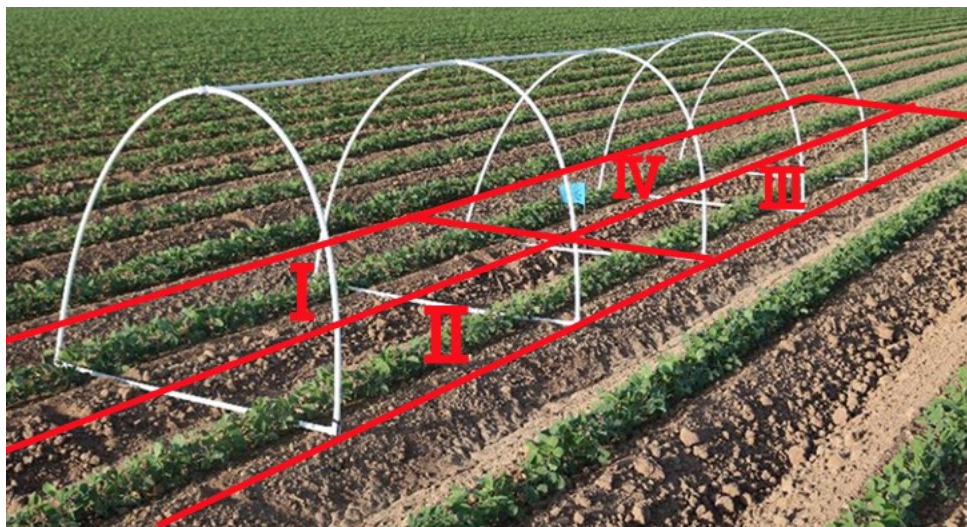


Figure 3.1 PVC frame of low-tunnel tent with quadrant diagram



Figure 3.2 Completed low-tunnel tent with contractor plastic covering

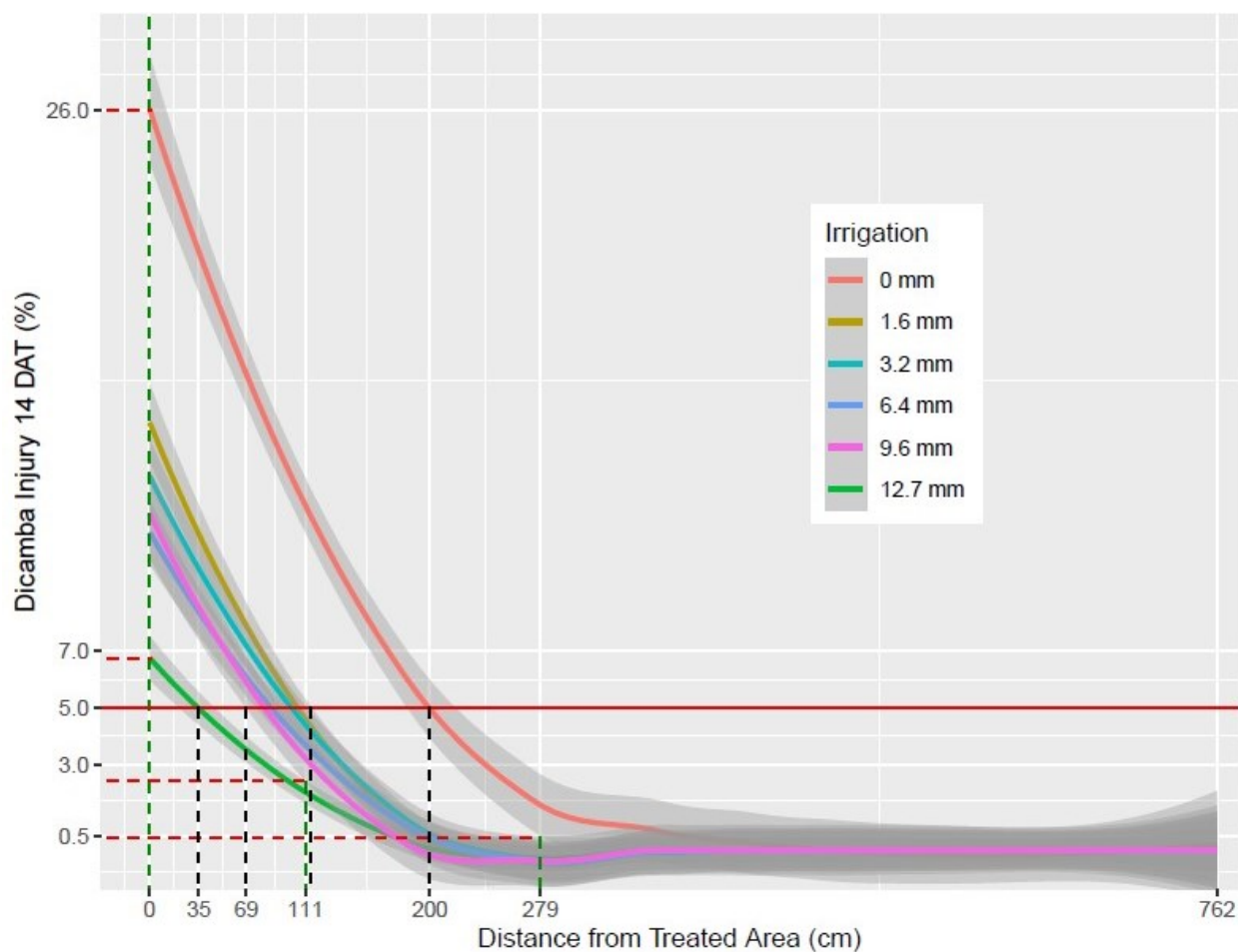


Figure 3.3 Effect of simulated irrigation quantity on dicamba vapor injury of non-DR soybean regressed over distance fourteen days after treatment<sup>ab</sup>

<sup>a</sup>Mean injury (%) non-linearly regressed over distance (cm); shaded area represents 95% confidence interval; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent % injury at injury separation; Vertical dashed green lines represent distance at injury separation; Vertical dashed black lines represent distance of 5% injury observation.

<sup>b</sup>Application of diglycolamine salt of dicamba at 2.24 kg ae ha<sup>-1</sup> + potassium salt of glyphosate at 4.49 kg ae ha<sup>-1</sup> with a carrier volume of 140 L ha<sup>-1</sup> followed by overhead irrigation

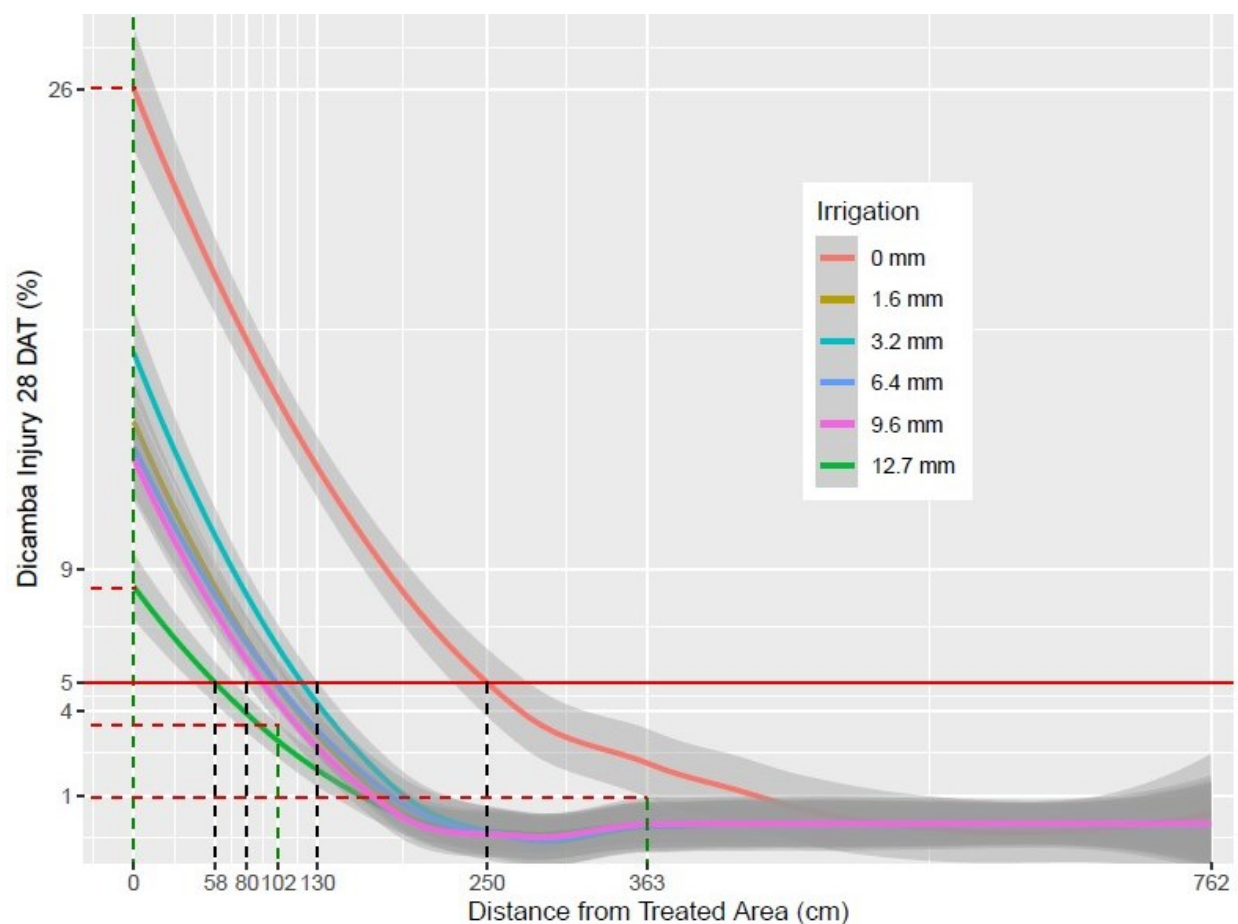


Figure 3.4 Effect of simulated irrigation quantity on dicamba vapor injury of non-DR soybean regressed over distance twenty-eight days after treatment<sup>ab</sup>

<sup>a</sup>Mean injury (%) non-linearly regressed over distance (cm); shaded area represents 95% confidence interval; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent % injury at injury separation; Vertical dashed green lines represent distance at injury separation; Vertical dashed black lines represent distance of 5% injury observation.

<sup>b</sup>Application of diglycolamine salt of dicamba at 2.24 kg ae ha<sup>-1</sup> + potassium salt of glyphosate at 4.49 kg ae ha<sup>-1</sup> with a carrier volume of 140 L ha<sup>-1</sup> followed by overhead irrigation

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## CHAPTER IV

### DETERMINING EFFECT OF TANK-MIX PARTNER AND PREMIXING ON DICAMBA VOLATILITY

#### **Abstract**

Dicamba off-target-movement (OTM) has become more common with increased application frequency following introduction of DT crops. One form of dicamba OTM is volatility. Tank-mixed products can impact the volatile behavior of dicamba following application. Field experiments were conducted in 2018 to evaluate impact of tank-mixed products on dicamba volatility. Glyphosate salt evaluation consisted of treatments containing diglycolamine salt of dicamba with VaporGrip™ applied at 2.24 kg ae ha<sup>-1</sup> alone, dicamba plus DMA-glyphosate applied at 4.48 kg ae ha<sup>-1</sup>, and dicamba plus K-glyphosate applied at 4.48 kg ha<sup>-1</sup>. Evaluation of OTM mitigation agent consisted of three dicamba plus K-glyphosate treatments containing no drift reduction agent (DRA) or volatility reduction agent (VRA), a DRA applied as Intact® at 2% v/v, and a VRA applied as MON 10 at 4% v/v. Volatility comparison of tank mix and premix treatments consisted of dicamba plus K-glyphosate, MON 76981 – diglycolamine salt of dicamba plus monoethanolamine glyphosate plus VaporGrip® - at 6.73 kg ae ha<sup>-1</sup>, and MON 76981 applied at 6.73 kg ae ha<sup>-1</sup> plus glufosinate applied at 8.48 L ha<sup>-1</sup>. Herbicides were applied at 4X rates to ensure visual symptomology. Treatments were applied to greenhouse flats filled with field soil. Following application, treated flats were placed between two rows of non-DT soybean within plots. Both salts of glyphosate increased volatility while

DMA-glyphosate had the most volatile effect. Additions of any OTM mitigation agent decreased vapor injury with the addition of both a DRA and VRA having the most mitigating effect. No effect on volatility was observed between dicamba plus K-glyphosate and MON 76981 alone or with glufosinate.

**Nomenclature:**

Diglycolamine salt of dicamba; potassium salt of glyphosate; dimethylamine salt of glyphosate; glufosinate; volatility reduction agent; drift reduction agent; soybean, *Glycine max*.

**Key Words:**

Tank mix; volatility

**Introduction**

Reliance on glyphosate alone as a POST herbicide in glyphosate tolerant crops created difficulty in weed management after glyphosate resistance developed (Green 2018, Powles 2008). Today, 17 weed species have developed resistance to glyphosate (Heap and Duke 2018). Dicamba-resistant (DR) cropping systems have become one solution for the management of this growing resistance issue. Dicamba has proven to be effective in controlling glyphosate resistant weeds in POST applications (Johnson et al. 2010). Following release, adoption of DR crop technology occurred largely in regions experiencing widespread glyphosate-resistant weed populations (Weschler et al. 2019). In the 2018 Mississippi cropping season, 79% of soybean hectares were planted in dicamba tolerant varieties (Weschler et al. 2019). Expansion of dicamba use with rapid technology adoption led to increasing concern of off-target-movement (OTM) (Bradley 2017, Hager 2017, Steckel et al. 2017).

Cases of OTM became apparent soon after the 2016 DR crop release (EPA 2020). In the 2017 cropping season, roughly 2,708 cases of dicamba OTM were observed in U.S. soybean

producing regions, totaling roughly 1.5 million hectares of injured soybean (Bradley 2017, EPA 2020). In 2018, dicamba regulations were tightened to mitigate OTM (EPA 2020). Label changes outlined legal dicamba formulation, legal tank mix, droplet size requirement, application timing, drift reduction agent (DRA) use, and volatility reduction agent (VRA) use (EPA 2020). Despite tightening of application requirements, an increase in EPA reported OTM cases involving dicamba was observed from the 2018 to 2019 cropping seasons, increasing from 2,600 to 3,000 cases (EPA 2020).

OTM events of dicamba occur as physical drift, tank contamination, and volatility (Soltani et al. 2020, Behrens and Lueschen 1979, Boerboom 2004). Mitigation efforts of physical drift include use of low-drift nozzles, low-drift sprayer design, use of DRAs, reduction of ground speeds, and making applications under favorable weather conditions (Foster et al. 2018, Creech et al. 2015, Womac et al. 1997). Dicamba contamination of spray equipment can be mitigated through segregation of equipment by herbicide technology, or selection of sprayer components less likely to sequester the herbicide (Cundiff et al. 2017). Vapor movement mitigation efforts include use of a VRA, monitoring weather conditions, timing of application, and understanding tank mix effect (Behrens and Lueschen 1979, Mueller et al. 2013, EPA 2020). Understanding tank-mixing effect on volatility allows for applicator-controlled mitigation efforts before the sprayer enters the field.

Tank-mixing has become a popular method to mitigate resistance, broaden spectrum of control, and reduce the number of herbicide applications (Beckie and Reboud 2009, Norsworthy et al. 2012). With dicamba's lack of activity on grass species and limited residual activity, POST dicamba applications routinely include a tank mix partner with additional activity (Werle et al. 2018, Spaunhorst and Bradley 2013). With DR cropping systems also exhibiting glyphosate

tolerance and some cultivars expressing glufosinate tolerance, these herbicides are frequently included for additional control of susceptible broadleaf species and grasses (Werle et al. 2018). In a 2018 survey in Nebraska, 60% of producers applied dicamba alone or with glyphosate POST, while 40% applied dicamba with other MOAs (Werle et al. 2018). Additional products can affect the behavior of dicamba volatility after application (Mueller and Steckel 2019a, 2019b).

VaporGrip® technology was introduced as the “in-the-jug” volatility mitigation agent and is present in the diglycolamine salt of dicamba (DGA) formulations applied in DT crops (Anonymous 2019, Anonymous 2020). VaporGrip® is designed to reduce the formation of free dicamba acid in spray solutions by acting as a buffering agent using acetic acid (Abraham 2018, MacInnes 2017). Dissociated dicamba acid is more volatile than dicamba formulated as a salt (Behrens and Lueschen 1979). Acetic acid present in the technology binds up free dicamba ions dissociating in solution before dicamba acid formation can occur (Abraham 2018). A 2020 study found that VaporGrip® technology in tank mix with DGA salt of dicamba reduced volatility injury compared to DGA salt of dicamba alone and N,N-Bis-(3-aminopropyl) methylamine salt (BAPMA) salt of dicamba (Oseland et al. 2020). Tightening of application requirements has also included use of additional VRAs and DRAs in tank-mixing of certain herbicides, including glyphosate (Anonymous 2019, Anonymous 2020). Tank-mixing regulation has been everchanging throughout herbicide registration in DT crops (EPA 2020).

Additional products are being developed to further assist in the mitigation of dicamba volatility. MON 10, an experimental VRA, has been developed for dicamba volatility mitigation. MON 76981 – a pre-mix product containing diglycolamine salt of dicamba plus monoethanolamine salt of glyphosate + VaporGrip® was developed as a premix option offering

both dicamba and glyphosate in a formulated product. MON 76981 is intended to increase ease of use and possibly decrease cost of POST applications containing these active ingredients. Research will increase understanding of the behavior of dicamba with commonly tank mixed herbicides, DRA's, VRA's, and in pre-mixed products to further improve farm-level mitigation efforts by applicators.

### **Materials and Methods**

Experiments were conducted in 2018 at the R.R. Foil Research Station in Starkville, MS with additional locations in Guelph, ON, Moultrie, GA, Macomb, IL, Lafayette, IN, Alexandria, LA, East Lansing, MI, North Platte, NE, College Station, TX, and Arlington, WI. Experiments evaluated the effect of glyphosate salts, DRAs, VRAs, and pre-mixed dicamba products on dicamba volatility. Treatments related to glyphosate salt evaluation consisted of diglycolamine salt of dicamba with VaporGrip® applied at 2.24 kg ae ha<sup>-1</sup> alone, dicamba plus dimethylamine salt of glyphosate (DMA) applied at 4.48 kg ae ha<sup>-1</sup>, and dicamba plus potassium salt of glyphosate (K) applied at 4.48 kg ha<sup>-1</sup>. Evaluation of OTM mitigation agent consisted of three dicamba plus K treatments containing no DRA or VRA, DRA applied as Intact® at 2% v/v, and a VRA applied as MON 10 at 4% v/v. Volatility comparison of tank mix and premix treatments consisted of dicamba plus K, MON 76981 – a pre-mixed product containing diglycolamine salt of dicamba plus monoethanolamine salt of glyphosate plus VaporGrip® - at 6.73 kg ae ha<sup>-1</sup>, and MON 76981 applied at 6.73 kg ae ha<sup>-1</sup> plus glufosinate applied at 8.48 L ha<sup>-1</sup>. Applications were made by a single sprayer pass using 4X herbicide rates with a carrier volume of 140 L ha<sup>-1</sup> to ensure symptomatic response on indicator soybean. Treatments were applied to soil-filled greenhouse flats (Heavy Duty 1020 Tray, Greenhouse Megastore) kept weed free and uniform in

surface texture. Soil flats were transported to the experiment site via truck bed following herbicide application.

Plots measured 15.3 x 0.8 m, with replications separated by an alley measuring 6.1m. Two unplanted rows measuring 2.3 m in width separated plots within the same replication to mitigate potential vapor movement between plots. Two rows of non-DR soybean were planted to act as indicator plants within plots. Treatment occurred between the V4 and V5 vegetative soybean growth stages to ensure dicamba exposure prior to initiation of reproductive structures. A 1.5 x 4.6 m PVC frame was placed in the center of each plot and treated soil flats placed beneath (Figure 3.1). Following flat transportation and placement within plots, contractor's plastic was draped over the PVC structure and clamped on both ends (Figure 3.2). Ends of the low tunnel remain open to allow for air movement through the tunnel. Treated greenhouse flats and low tunnels remained in each plot for a 48-hour exposure period. Following the 48-hour exposure period, all low tunnels, contractor's plastic, and soil flats were removed from the field.

Collection of visible injury and plant heights occurred 14 and 28 days after treatment (DAT). Evaluations began following the conclusion of the 48-hour exposure period. One to two days prior to the first evaluation, the most injured quadrant of each plot was identified (Figure 3.1). Evaluations occurred within this quadrant at both intervals. Ratings in the selected quadrant occurred outward from the center of the plot in 30 cm intervals. Plant injury ratings used a scale from 0 to 100% as a percentage of injury compared to the untreated check (Behrens and Leuschen 1979). Plant heights were collected in centimeters at each rating interval.

Data analysis used SAS 9.4 (SAS Institute Inc, Cary, NC) and RStudio (RStudio Inc, Boston, MA). Data were subjected to ANOVA to determine significance and interactions among factors. Comparison of injury ratings and plant height at each rating interval individually used

PROC GLIMMIX with means separated by LSMEANS using an alpha level of 0.05 in SAS 9.4 software. Plant injury and plant height data were also nonlinearly regressed over site year with a 95% confidence band using the loess package in Rstudio due to non-parametric behavior of the data (Scholtes et al. 2019).

## **Results**

Data analysis indicated no differences across locations; therefore, data were pooled over location. Differences among treatments were observed. Plant injury data are presented with related treatments to evaluate glyphosate salt effect, effect of DRA and VRA, and premixing effects. Plant height was unaffected.

Injury data analyzed by evaluation distance 14 DAT found DMA-glyphosate produced more vapor injury than treatments containing K-glyphosate and no glyphosate from 0 to 671 cm (Table 4.1). Treatments containing no glyphosate produced the least vapor injury among treatments at evaluation distances between 0 and 305 cm 14 DAT (Table 4.1). When 14 DAT vapor injury was regressed over distance, DMA-glyphosate was the most injurious of the glyphosate salt additions from 0 to 486 cm (Figure 4.3). Injury through this separation ranged from 31% at 0 cm to 5% at 454 cm (Figure 4.3). When regressed over distance 14 DAT, treatments containing no glyphosate produced the least vapor injury from 0 to 352 cm, with injury ranging from 17% at 0 cm and falling to 5% at 174 cm (Figure 4.3). Treatments containing K-glyphosate produced more vapor injury than treatments containing no glyphosate from 0 to 352 cm but were still less injurious than those containing DMA-glyphosate 14 DAT (Figure 4.3). Vapor injury from treatments containing K-glyphosate ranged from 23% at 0 cm and fell to 5% at 284 cm 14 DAT (Figure 4.3).

Glyphosate salt effect on vapor injury analyzed by evaluation distance 28 DAT revealed an increase in vapor injury with the presence of DMA-glyphosate at evaluation distances between 0 and 610 cm (Table 4.2). At 0 and 30 cm 28 DAT, no difference in injury was observed from treatments containing K-glyphosate and no glyphosate (Table 4.2). At each evaluation distance between 61 and 274 cm and at 366 cm 28 DAT, treatments lacking any glyphosate produced less vapor injury than treatments containing either glyphosate addition (Table 4.2). Vapor injury regressed over distance 28 DAT found the presence of DMA-glyphosate produced the highest vapor injury levels from 0 to 459 cm (Figure 4.4). Treatments containing DMA-glyphosate produced injury of 30% at 0 cm and fell to 5% at 424 cm (Figure 4.4). Treatments lacking glyphosate produced the least vapor injury from 0 to 325 cm 28 DAT, with injury below 16% at 0 cm and decreased to 5% at 159 cm (Figure 4.4). Treatments containing K-glyphosate again produced more vapor injury than treatments containing no glyphosate from 0 to 325 cm 28 DAT but were still less injurious than treatments containing DMA-glyphosate (Figure 4.4). Vapor injury from treatments containing K-glyphosate were above 18% at 0 cm and fell to 5% at 247 cm 28 DAT (Figure 4.4).

Treatments containing at least one OTM mitigation agent decreased vapor injury at evaluation distances from 0 to 488 cm when compared to treatments lacking both (Table 4.3). Treatments containing a VRA produced the least vapor injury of all three treatments at 61 cm and at evaluation distances from 152 to 244 cm 14 DAT (Table 4.3). Vapor injury regressed over distance 14 DAT found the presence of both OTM mitigation agents produced the least vapor injury from 17 to 252 cm, with injury between 15 and 16% at 17 cm and falling to 5% at 134 cm (Figure 4.5). Treatments lacking both agents produced the most vapor injury from 0 to 394 cm 14 DAT, with injury of 23% at 0 cm and falling to 5% at 283 cm (Figure 4.5). Treatments



containing only the DRA product resulted in higher injury when compared to treatments containing an additional VRA product from 17 to 252 cm 14 DAT, with injury of roughly 18% at 0 cm and falling to 5% at 176 cm (Figure 4.5). Treatments containing only the DRA product still produced less vapor injury than treatments lacking both OTM mitigation agents through this range (Figure 4.5).

Treatments containing at least one OTM mitigation agent decreased vapor injury at each evaluation distance from 61 to 396 cm 28 DAT when compared to treatments lacking both (Table 4.4). At evaluation distances between 91 and 274 cm 28 DAT, the presence of a VRA reduced vapor injury when compared to treatments containing only a DRA and those lacking both agents (Table 4.4). When injury data from these treatments was regressed over distance 28 DAT, treatments lacking both OTM mitigation agents produced the most severe vapor injury response from 0 and 324 cm, with injury of approximately 19% at 0 cm decreasing to 5% at 246 cm (Figure 4.6). Treatments containing both OTM mitigation agents produced the least vapor injury from 8 to 283 cm when regressed over distance 28 DAT, with injury of roughly 14% at 8 cm that fell to 5% at 121 cm (Figure 4.6). Treatments containing only a DRA product resulted in higher injury from 0 to 283 cm when compared to treatments containing an additional VRA product, but were less injurious than treatments lacking both products through this range (Figure 4.6)

No difference in vapor injury at any evaluation distance was observed among either treatment containing MON 76981 or the treatment containing dicamba + K-glyphosate at either evaluation timing (Table 4.5, Table 4.6). When vapor injury of these related treatments was regressed over distance, no separation was observed at any distance at either evaluation (Figure 4.7, Figure 4.8). Five percent or greater vapor injury was observed to distances between 255 and

347 cm regardless of pre-mix or tank mixture 14 DAT (Figure 4.7). At 28 DAT, 5% or greater injury was observed to distances between 222 and 277 cm (Figure 4.8).

These data support current regulation regarding the required use of VRAs to mitigate volatility when making tank mixed applications and suggest DRAs may assist in volatility mitigation. Data also support the prohibition of applying DMA salt of glyphosate with current dicamba products. Data suggest that no difference between volatility exists between mixing of products in-tank and applying a pre-mixed product in MON 76981.

Table 4.1 Effect of glyphosate salt on dicamba vapor injury response by evaluation distance fourteen days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup>	Glyphosate Salt Tank Mix <sup>c</sup>		
	DMA Glyphosate	K Glyphosate	No Glyphosate
(cm)	-----	% -----	-----
0	32 a	23 b	18 c
30	30 a	20 b	15 c
61	27 a	18 b	12 c
91	26 a	16 b	9 c
122	24 a	13 b	8 c
152	23 a	12 b	5 c
183	22 a	10 b	5 c
213	20 a	8 b	3 c
244	18 a	7 b	2 c
274	17 a	6 b	2 c
305	15 a	4 b	1 c
335	11 a	2 b	1 b
366	10 a	2 b	1 b
396	8 a	2 b	1 b
427	6 a	1 b	0 b
457	4 a	2 b	0 b
488	3 a	1 b	1 b
518	3 a	1 b	1 b
549	2 a	0 b	0 b
579	2 a	0 b	0 b
610	1 a	0 b	0 b
640	1 a	0 b	0 b
671	1 a	0 b	0 b
701	1 a	0 a	0 a
732	1 a	0 a	0 a
762	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent differences of vapor injury at each individual rating interval represented in table rows

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>DMA Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> DMA salt of glyphosate; K Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; No Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba

Table 4.2 Effect of glyphosate salt on dicamba vapor injury response by evaluation distance twenty-eight days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup>	Glyphosate Salt Tank Mix <sup>c</sup>		
	DMA Glyphosate	K Glyphosate	No Glyphosate
(cm)	-----	% -----	-----
0	31 a	19 b	17 b
30	28 a	16 b	14 b
61	28 a	14 b	10 c
91	25 a	11 b	7 c
122	24 a	10 b	6 c
152	23 a	9 b	5 c
183	20 a	7 b	4 c
213	19 a	6 b	3 c
244	17 a	5 b	2 c
274	16 a	5 b	2 c
305	14 a	3 b	1 b
335	9 a	2 b	1 b
366	7 a	2 b	1 c
396	6 a	2 b	1 b
427	4 a	0 b	0 b
457	3 a	0 b	0 b
488	3 a	0 b	0 b
518	2 a	0 b	0 b
549	2 a	0 b	0 b
579	1 a	0 b	0 b
610	1 a	0 b	0 b
640	1 a	0 ab	0 b
671	0 a	0 a	0 a
701	0 a	0 a	0 a
732	0 a	0 a	0 a
762	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent differences of vapor injury at each individual rating interval represented in table rows

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>DMA Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> DMA salt of glyphosate; K Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; No Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba

Table 4.3 Effect of VRA and DRA on dicamba vapor injury response by evaluation distance fourteen days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup>	OTM Mitigating Agent <sup>c</sup>		
	No DRA or VRA	DRA	DRA + VRA
(cm)	-----	% -----	-----
0	23 a	18 b	17 b
30	20 a	16 b	14 b
61	18 a	15 b	9 c
91	16 a	9 b	7 b
122	13 a	8 b	5 b
152	12 a	5 b	3 c
183	10 a	5 b	2 c
213	8 a	3 b	1 c
244	7 a	2 b	1 c
274	6 a	1 b	0 b
305	4 a	1 b	1 b
335	2 a	1 b	0 b
366	2 a	0 b	0 b
396	2 a	0 b	0 b
427	1 a	0 b	0 b
457	2 a	0 b	0 b
488	1 a	0 b	0 b
518	1 a	1 ab	0 b
549	0 a	0 a	0 a
579	0 a	0 a	0 a
610	0 a	0 a	0 a
640	0 a	0 a	0 a
671	0 a	0 a	0 a
701	0 a	0 a	0 a
732	0 a	0 a	0 a
762	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent differences of vapor injury at each individual rating interval represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>No VRA - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; DRA – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact; DRA+ VRA - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact + 4% v/v MON 10

Table 4.4 Effect of VRA and DRA on dicamba vapor injury response by evaluation distance twenty-eight days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup>	OTM Mitigating Agent <sup>c</sup>		
	No DRA or VRA	DRA	DRA + VRA
(cm)	-----	% -----	
0	20 a	17 ab	15 b
30	16 a	14 ab	12 b
61	14 a	10 b	7 b
91	11 a	8 b	5 c
122	10 a	7 b	4 c
152	9 a	6 b	3 c
183	7 a	4 b	2 c
213	6 a	3 b	1 c
244	5 a	3 b	1 c
274	5 a	2 b	1 c
305	3 a	2 b	1 b
335	2 a	1 b	0 b
366	2 a	1 b	0 b
396	2 a	0 b	0 b
427	0 a	0 a	0 a
457	1 a	0 a	0 a
488	0 a	0 a	0 a
518	0 a	0 a	0 a
549	0 a	0 a	0 a
579	0 a	0 a	0 a
610	0 a	0 a	0 a
640	0 a	0 a	0 a
671	0 a	0 a	0 a
701	0 a	0 a	0 a
732	0 a	0 a	0 a
762	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent differences of vapor injury at each individual rating interval represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>No VRA - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; DRA – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact; DRA + VRA - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact + 4% v/v MON 10

Table 4.5 Effect of dicamba mix on injury response by evaluation distance fourteen days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Dicamba Mix <sup>c</sup>		
	Dicamba + K	MON 76981	MON 76980 + Glufosinate
	-----	% -----	
0	23 a	24 a	22 a
30	20 a	20 a	21 a
61	18 a	16 a	18 a
91	16 a	15 a	16 a
122	13 a	15 a	15 a
152	12 a	12 a	12 a
183	10 a	10 a	11 a
213	8 a	8 a	8 a
244	7 a	7 a	8 a
274	6 a	5 a	6 a
305	4 a	4 a	5 a
335	2 a	4 a	4 a
366	2 a	2 a	3 a
396	2 a	2 a	3 a
427	1 a	1 a	2 a
457	2 a	1 a	2 a
488	1 a	1 a	1 a
518	1 a	1 a	1 a
549	0 a	1 a	1 a
579	0 a	1 a	0 a
610	0 a	0 a	1 a
640	0 a	0 a	0 a
671	0 a	0 a	0 a
701	0 a	0 a	0 a
732	0 a	0 a	0 a
762	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent differences of vapor injury at each individual rating interval represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>Dicamba + K – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; MON 76981 – 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate; MON 76981 + Glufosinate - 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate + 8.48 L ha<sup>-1</sup> glufosinate

Table 4.6 Effect of dicamba mix on injury response by evaluation distance twenty-eight days after treatment<sup>a</sup>

Distance from treated soil <sup>b</sup> (cm)	Dicamba Mix <sup>c</sup>		
	Dicamba + K	MON 76981	MON 76980 + Glufosinate
	-----	% -----	
0	19 a	21 a	19 a
30	16 a	19 a	18 a
61	14 a	16 a	15 a
91	11 a	13 a	14 a
122	10 a	11 a	12 a
152	9 a	9 a	9 a
183	7 a	8 a	7 a
213	6 a	7 a	6 a
244	5 a	5 a	5 a
274	5 a	5 a	4 a
305	3 a	4 a	3 a
335	2 a	1 a	2 a
366	2 a	1 a	2 a
396	2 a	1 a	2 a
427	0 a	1 a	1 a
457	1 a	1 a	1 a
488	0 a	1 a	1 a
518	0 a	1 a	0 a
549	0 a	1 a	0 a
579	0 a	0 a	0 a
610	0 a	0 a	0 a
640	0 a	0 a	0 a
671	0 a	0 a	0 a
701	0 a	0 a	0 a
732	0 a	0 a	0 a
762	0 a	0 a	0 a

<sup>a</sup>Means averaged over locations separated by LSMEANS ( $\alpha=0.05$ ). Letters represent differences of vapor injury at each individual rating interval represented in table rows.

<sup>b</sup>Distances rounded to nearest cm

<sup>c</sup>Dicamba + K – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; MON 76981 – 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate; MON 76981 + Glufosinate - 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate + 8.48 L ha<sup>-1</sup> glufosinate



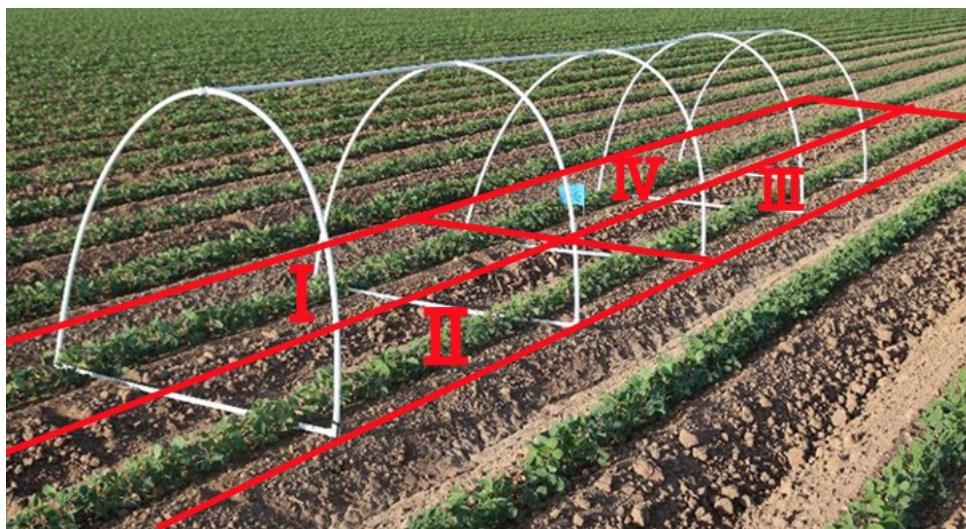


Figure 4.1 PVC frame of low-tunnel tent with quadrant diagram



Figure 4.2 Completed low-tunnel tent with contractor plastic covering

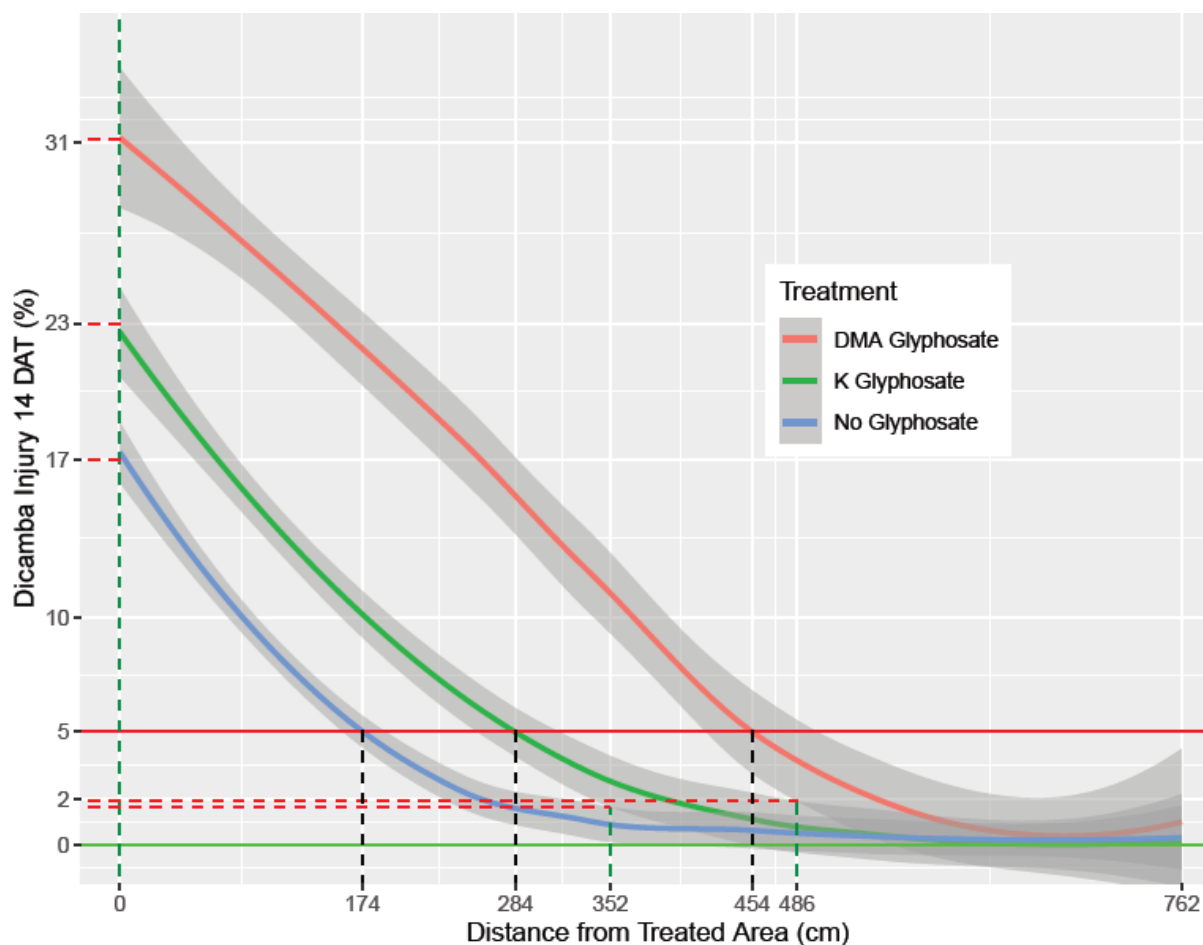


Figure 4.3 Effect of glyphosate salt on dicamba vapor injury of non-DR soybean regressed over distance fourteen days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>DMA Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> DMA salt of glyphosate; K Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; No Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba

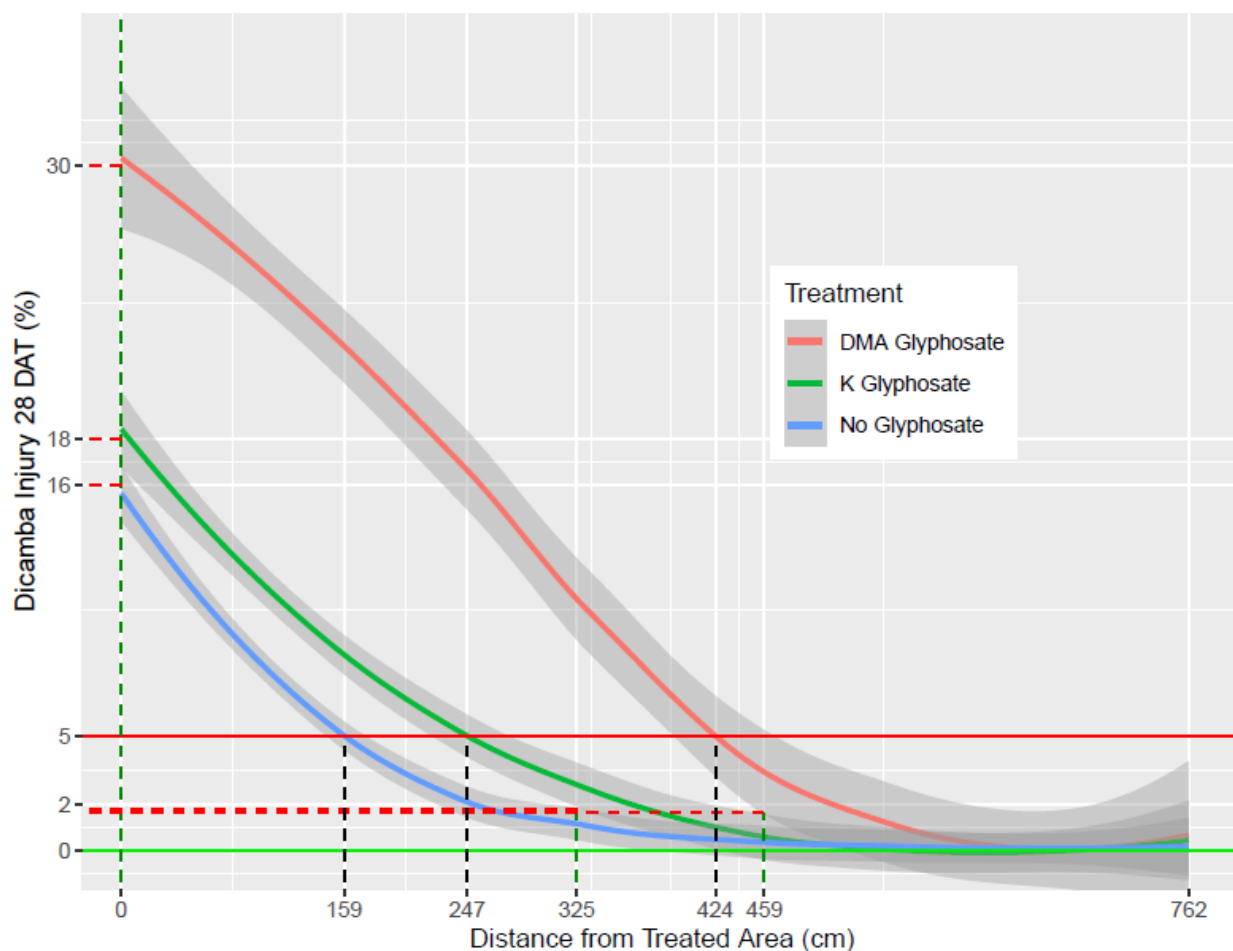


Figure 4.4 Effect of glyphosate salt on dicamba vapor injury of non-DR soybean regressed over distance twenty-eight days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>DMA Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> DMA salt of glyphosate; K Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; No Glyphosate – 2.24 kg ae ha<sup>-1</sup> dicamba

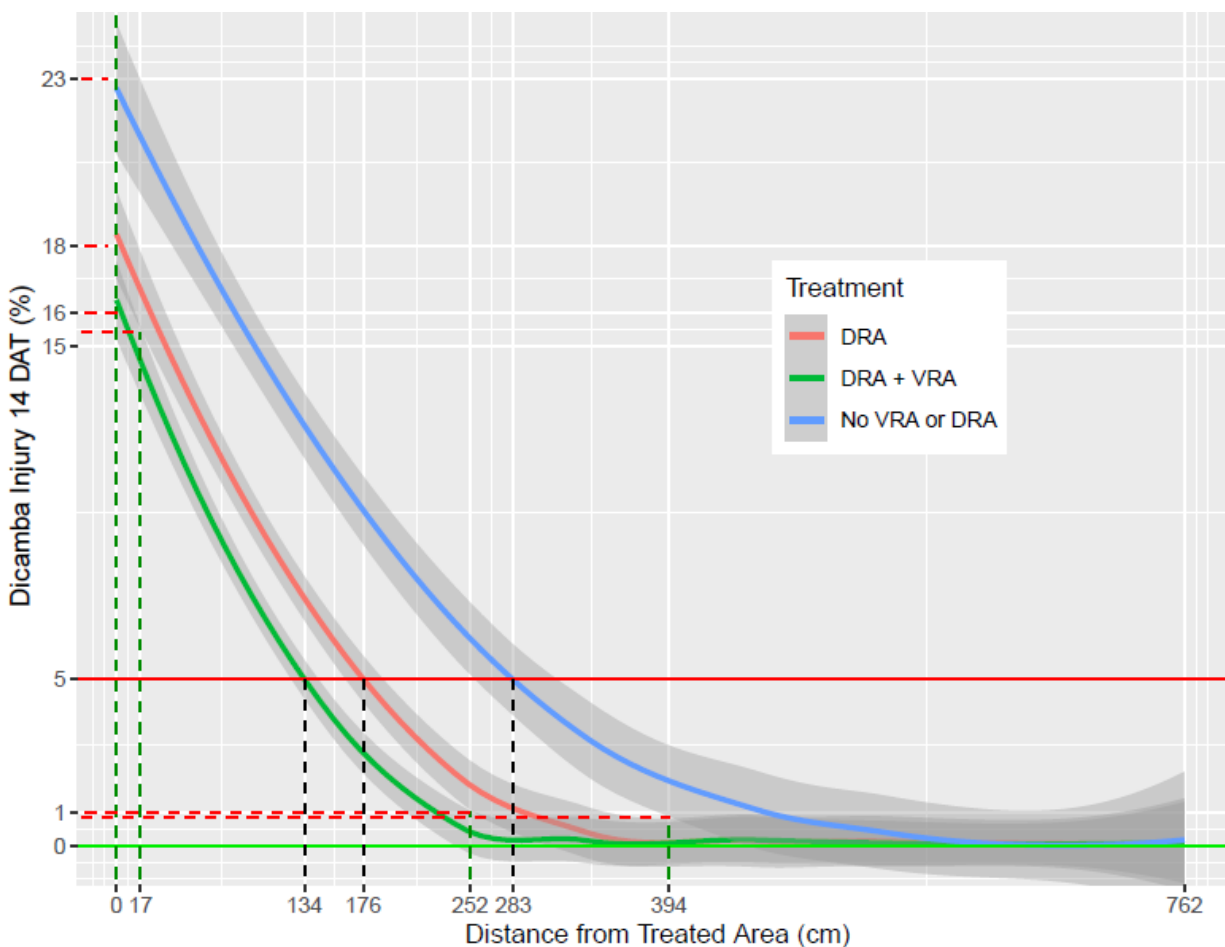


Figure 4.5 Effect of OTM mitigation agent on dicamba vapor injury of non-DR soybean regressed over distance fourteen days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>No VRA - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; Intact - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact; MON 10 - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact + 4% v/v MON 10

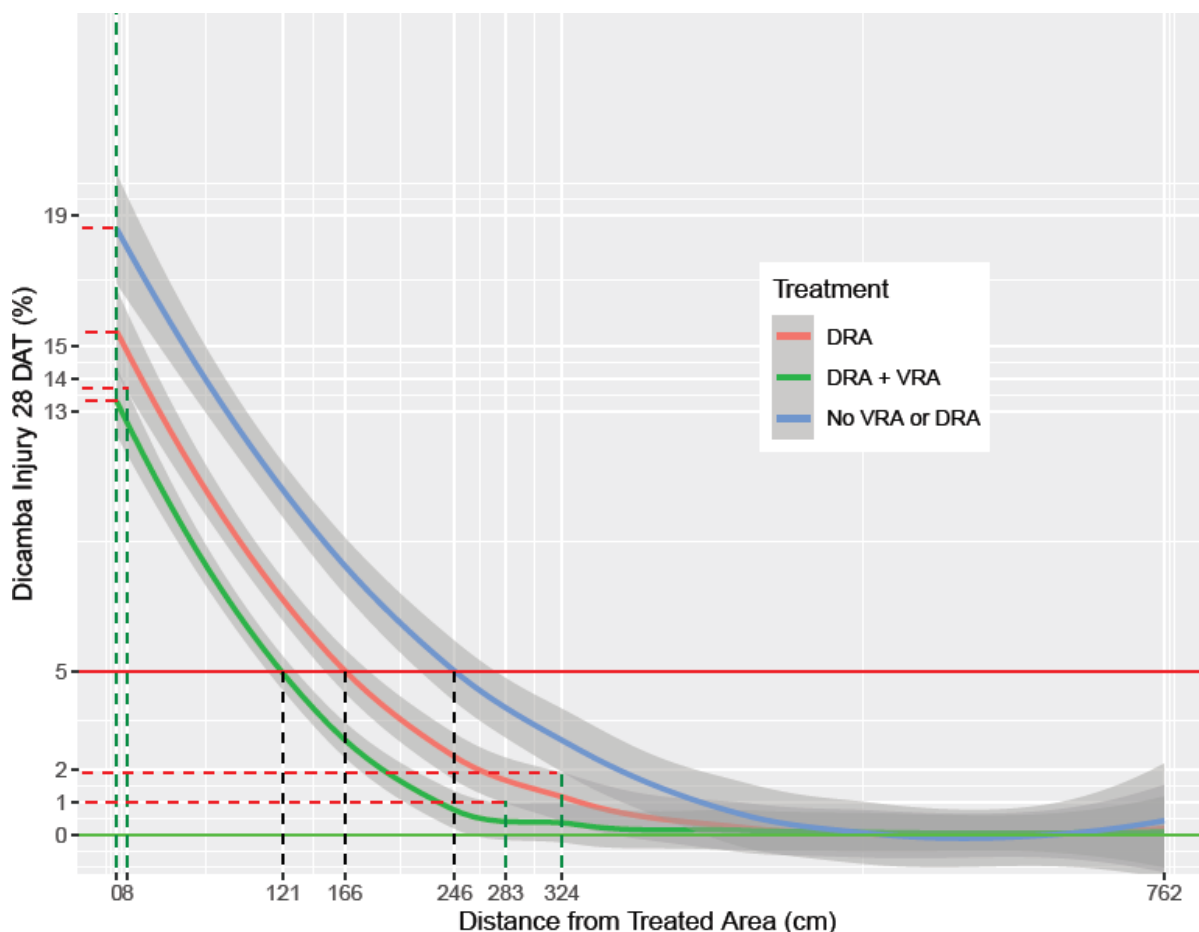


Figure 4.6 Effect of OTM mitigation agent on dicamba vapor injury of non-DR soybean regressed over distance twenty-eight days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>No VRA - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; Intact - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact; MON 10 - 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate + 2% v/v Intact + 4% v/v MON 10

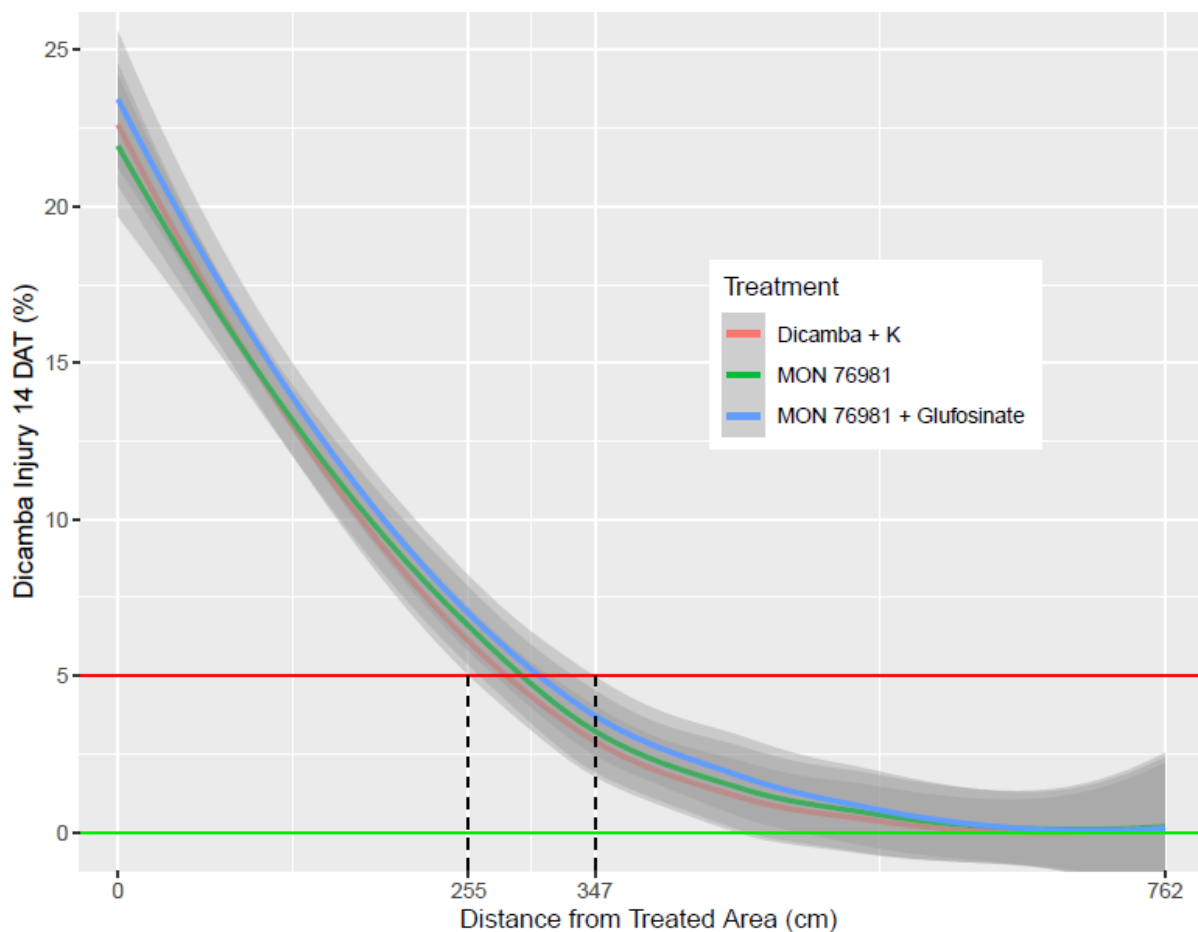


Figure 4.7 Effect of tank mix and premix on dicamba vapor injury of non-DR soybean regressed over distance fourteen days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>Dicamba + K – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; MON 76981 – 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate; MON 76981 + Glufosinate – 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate + 8.48 L ha<sup>-1</sup> glufosinate

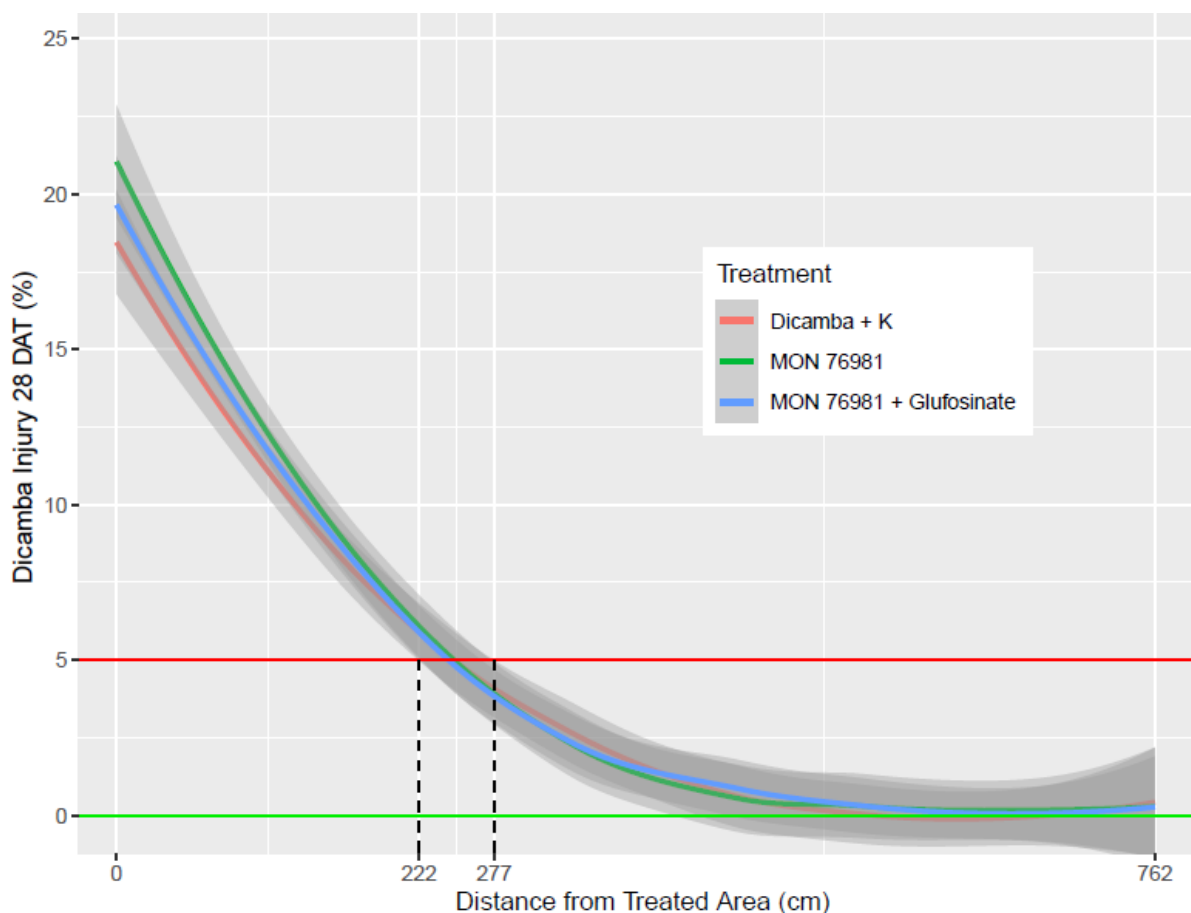


Figure 4.8 Effect of tank mix and premix on dicamba vapor injury of non-DR soybean regressed over distance twenty-eight days after treatment<sup>abc</sup>

<sup>a</sup>Vapor injury (%) non-linearly regressed over distance (cm)

<sup>b</sup>Grey shaded area represents 95% confidence interval; Horizontal solid green line represents no injury; Horizontal solid red line represents 5% injury; Horizontal dashed red lines represent injury % at separation; Vertical dashed green lines represent distance at separation; Vertical dashed black lines represent distance of 5% injury observation

<sup>c</sup>Dicamba + K – 2.24 kg ae ha<sup>-1</sup> dicamba + 4.48 kg ae ha<sup>-1</sup> K salt of glyphosate; MON 76981 – 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate; MON 76981 + Glufosinate – 6.73 kg ae ha<sup>-1</sup> premix of dicamba with K salt of glyphosate + 8.48 L ha<sup>-1</sup> glufosinate



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